

## Scotland's Rural College

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811 **Pea cultivar and wheat residues affect carbon/nitrogen dynamics in pea-triticale**  
812 **intercropping: a microcosms approach**

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822

823 **Abstract**

824 The underlying mechanisms by which legume cultivars contribute to nitrous oxide (N<sub>2</sub>O) generation  
825 are poorly understood. The aim of the present study was to explore the effects of two pea cultivars  
826 (Zero4 and Nitouche) intercropped with triticale, with or without wheat (*Triticum aestivum*) residues  
827 incorporation, on soil C and N dynamics, on bacterial community structure and their links with N<sub>2</sub>O  
828 emissions. Monocrops and bare soil (no plant) treatments were used as an additional control in order  
829 to account for the level of mineralisation between treatments. Changes in total C and N contents and  
830 in some functionally-related soil pools (microbial biomass C and N, basal respiration, KCl-  
831 exchangeable ammonium and nitrate, potentially mineralizable N, DOC, ecophysiological indexes)  
832 were followed throughout a 97-day microcosm experiment carried out on a loamy arable soil.  
833 ARISA community fingerprinting of soil extracted DNA and GHG emissions were carried out at  
834 two key stages (pea flowering and harvest). The addition of residues to the soil resulted in only small  
835 changes to the total C and N pools the Nitouche monocrop, which was found to have the highest  
836 potentially mineralisable N ( $13.4 \mu\text{g g}^{-1} 28\text{d}^{-1}$ ) of the treatments with added residue. The different  
837 pea cultivar selectively affected N<sub>2</sub>O emissions, with highest emissions associated with the cultivar  
838 Nitouche in the absence of residues. The two intercropping treatments of triticale/pea were  
839 significantly different either with residues or without, especially the triticale/Zero 4 which had the  
840 lowest values ( $356 \text{ g N}_2\text{O-N ha}^{-1}$ ). Similar patterns were also observed in below ground data. ARISA  
841 analysis showed that monocropped legumes and the Triticale-based treatment clearly grouped on  
842 separate clusters to the added residue treatment. We hypothesize that in pea-based intercrops  
843 variations in carbon supply from different cultivars may contribute to differences in N<sub>2</sub>O emissions  
844 and thus influence the choice of suitable cultivars, to optimize nutrient cycling and sustainable crop  
845 management.

846 **Keywords**

847 bacterial community structure, C and N pools, N<sub>2</sub>O emissions, pea-based intercropping, wheat

848 residues

849 **Introduction**

850 Legume cropping offers opportunities to reduce GHG emissions from agriculture through their  
851 ability to substitute inputs of mineral fertilisers with biologically fixed N (Rochette *and* Janzen  
852 2005). However, legumes differ widely in their contribution to N<sub>2</sub>O emissions and in some cases  
853 (particularly following residue incorporation) can still remain a significant source (Baggs *et al.*,  
854 2000; Bouwman *et al.*, 2002). The cultivation of leguminous crops in agricultural systems can not  
855 only contribute to reducing the emission of nitrous oxide (N<sub>2</sub>O) but also increases the release and  
856 the turnover of mineralisable N-containing compounds in soil (Rochette *and* Janzen 2005; Jensen *et*  
857 *al.*, 2010). Their ability to add external N to the plant-soil system is a distinct benefit on which crop  
858 production systems can rely on in order to maintain the soil N supply at a sustained productive level  
859 (Watson *et al.*, 2011). The amount of biologically fixed N supplied by legumes varies greatly from  
860 tens to several hundred kilograms per ha per year and is strongly affected by the type and  
861 environmental conditions (nitrate availability, temperature, soil wetness, and the availability of  
862 other nutrients).

863 Although symbiotic Rhizobium is believed to be able to produce N<sub>2</sub>O in root nodules there is a  
864 conflicting evidence regarding the magnitude of this process. In their early work, O'Hara and  
865 Daniel (1985) suggested that rhizobial microorganisms are directly involved in the production of  
866 N<sub>2</sub>O by reduction of NO<sub>3</sub> occurring within the root nodules. However, it is likely that Rhizobium  
867 species are not directly involved in the N<sub>2</sub>O production process, and that the root microflora also  
868 plays an important role. Okubo *et al.* (2009) have shown that the rhizosphere community structure  
869 is significantly influenced by plant species and cultivar. It is also likely that this community  
870 structure is influenced by environmental conditions. It has been shown that different nodulation  
871 phenotypes contain different bacterial and fungal profiles in the stems and roots (Ikeda *et al.*, 2008).  
872 However, the extent to which these phenotypes are associated with different emissions is unclear. In  
873 the case of legumes, it has been suggested that N<sub>2</sub>O emission is primarily associated with  
874 decomposition and turnover of root nodules (Inaba *et al.*, 2009), which implies that differences in  
875 the community structure and activity of root surface microorganisms may be responsible.

876 Understanding the contribution of legumes to N<sub>2</sub>O emissions in the wider environment is highly  
877 dependent on developing an improved understanding of the underlying microbiology of the system  
878 (Philippot *et al.*, 2002). Many studies have been conducted involving legume based cropping  
879 systems especially placed in intercrops or the growing of two or more species together at one time,  
880 since, legume-based intercropping is able to provide several agro-ecological services: a more  
881 efficient use of soil resources for plant growth due to a reduced competition for soil N (Hauggaard-  
882 Nielsen *et al.*, 2003; Knudsen *et al.*, 2004; Hauggaard-Nielsen and Jensen, 2005), an increased  
883 water and nutrient use efficiency (Hauggaard-Nielsen *et al.*, 2009a), a greater yield stability and  
884 higher N concentration in cereal grain (Hauggaard-Nielsen *et al.*, 2006, 2009b), a better control of  
885 soil erosion (Inal *et al.*, 2007), and an enhanced weed suppression and pest control (Liebman and  
886 Dyck, 1993; Corre-Hellou *et al.*, 2011). Moreover, reduced N<sub>2</sub>O emissions from soil (Pappa *et al.*,  
887 2011) were also shown in leguminous intercrops. One more justification for intercropping  
888 (especially pea-based) is the increased mineral N made available in the soil for the following crop  
889 (Pappa *et al.*, 2011; Scalise *et al.*, 2015). Finally, the legume cultivar has been shown to play an  
890 important role in the cumulative N<sub>2</sub>O emissions of the agricultural systems, which also affects the  
891 product intensities (Pappa *et al.*, 2011), which are all the emissions divided by all saleable outputs.

892 The aim of this study was to explore the mechanisms responsible for N<sub>2</sub>O emissions from two  
893 legume species demonstrated by Pappa *et al.* (2011) by monitoring a number of soil chemical (pH;  
894 EC; C<sub>org</sub>; Nt; NH<sub>4</sub><sup>+</sup>-N; NO<sub>3</sub><sup>-</sup>-N; DOC), biochemical (MBC; R<sub>bas</sub>; C<sub>0</sub>, potentially mineralisable C;  
895 MBC/C<sub>org</sub>; *q*M, mineralisation coefficient; *q*CO<sub>2</sub>; *q*CO<sub>2</sub>/C<sub>org</sub> ratio; MBN; PMN, potentially  
896 mineralisable N) variables together with the bacterial community structure  
897 by ARISA fingerprinting of soil extracted DNA, and GHGs emissions (N<sub>2</sub>O, CH<sub>4</sub>, CO<sub>2</sub>) in an  
898 arable soil as by a microcosms approach.

899 The present study tested the following three hypotheses: a) legume-based cropping systems and  
900 wheat residue incorporation can stimulate soil C and N cycling through the enhancement of the  
901 below-ground nutrient flow, b) GHG emissions from legume-based intercropping can be altered by

902 soil addition of wheat residues and c) even when showing a similar yield potential, the cultivar of a  
903 same leguminous species can selectively influence the soil processes including the bacterial  
904 community structure conditioned by the legume intercrop.

## 905 **2. Materials and methods**

### 906 *2.1 Soil type and plant material*

907 The soil used in the microcosm experiment was a loam collected from the Ap horizon (0-30 cm)  
908 of an agricultural field cultivated under continuous winter wheat and located at the Bush Estate,  
909 Edinburgh, Scotland (55°52'17.46" N, 3°12'24.27" W). The main soil properties were: sand 42%,  
910 silt 34%, clay 24%; bulk density  $1.2 \pm 0.1 \text{ kg dm}^{-3}$ ;  $\text{pH}_{\text{H}_2\text{O}}$   $6.19 \pm 0.04$ ; total organic C ( $\text{C}_{\text{org}}$ )  $34.27$   
911  $\pm 1.22 \text{ g kg}^{-1}$ ; total N ( $\text{N}_t$ )  $2.52 \pm 0.08 \text{ g kg}^{-1}$ ; C:N ratio  $13.62 \pm 0.20$ ;  $\text{NH}_4^+ - \text{N}$   $3.75 \pm 0.40 \text{ mg kg}^{-1}$ ;  
912  $\text{NO}_3^- - \text{N}$   $7.64 \pm 0.50 \text{ mg kg}^{-1}$ ; Olsen P  $18.2 \pm 0.4 \text{ mg kg}^{-1}$ ; extractable K  $202.0 \pm 0.3 \text{ mg kg}^{-1}$ ;  
913 electric conductivity measured in a soil:water (1:2, w/v) mixture ( $\text{EC}_{1:2}$  at  $25^\circ\text{C}$ )  $0.10 \pm 0.01 \text{ dS m}^{-1}$ .  
914 Following the winter wheat (*Triticum aestivum*) harvest (September 2011), residual straw was  
915 chopped to 2-4 mm and stored before being used for soil amendment. The soil for filling the  
916 microcosms was collected before starting the experiment (3<sup>rd</sup> October 2011), coarse sieved at < 4.7-  
917 mm particle size and brought to approximately 30% gravimetric water content. Seeds of two  
918 cultivars of spring pea (*Pisum sativum* L. cv. Nitouche and *Pisum sativum* L. cv. Zero4) were  
919 provided by PGRO (UK); seeds of triticale (*Triticum aestivum* L. x *Triticosecàle* Wittm.) were  
920 provided by APSOVSEMENTI s.p.a. (Pavia, I).

### 921 *2.2 Experimental set-up*

922 The microcosm study was carried out at Scotland's Rural College (SRUC), in Edinburgh,  
923 between October 2011 and February 2012. Microcosm units consisted of 2.12 L polyvinyl chloride  
924 (PVC) pipes (25 cm height, 10.4 cm internal diameter) that had been closed at the base with an air-  
925 tight seal using a sheet of Plexiglas<sup>®</sup>. A sampling point for the gas collection (a three-way tap) was  
926 placed at 23 cm depth from the surface of the microcosm. Microcosms were filled either with soil

927 (no residue addition) (unamended) or with a soil plus chopped wheat straw (400:1, w/w) mixture  
928 (corresponding to a 6.3 t ha<sup>-1</sup> addition rate at a field scale) (wheat residue addition) (amended).

929 The amount of soil needed was calculated by taking into account the microcosm volume  
930 (1867.92 cm<sup>3</sup>), the soil bulk density and the gravimetric water content in order to reach a water-  
931 filled pore space (WFPS) equal to 28-32% that provides optimum conditions for biological activity  
932 in soil (FAO, 2001). WFPS was kept constant during the growing season by watering with a N-free  
933 artificial rainwater (Palmqvist and Dahlman, 2006) in order to maintain suitable conditions for plant  
934 growth and microbial processes without providing an external N addition.

935 Soon after filling (7<sup>th</sup> October 2011), each microcosm, four seeds were initially sown but only  
936 two plants, of the same species or one of each intercrop components, were kept after successful seed  
937 germination. For each level of soil amendment, the following six treatments compared different  
938 combinations of leguminous intercrops and the respective sole crop: i) Nitouche: monocrop of pea  
939 cv. Nitouche; ii) Zero4: monocrop of pea cv. Zero4; iii) Triticale: monocrop of Triticale; iv)  
940 Nitouche-Triticale: intercrop pea cv. Nitouche-Triticale; v) Zero4-Triticale: intercrop pea cv. Zero4-  
941 Triticale and vi) bare soil: unplanted microcosms were used as a control.

942 Since the scheduled samplings were destructive, the whole experiment was duplicated, giving a  
943 total of 72 microcosms: (6 treatments) x (2 levels of amendment) x (2 samplings) x (3 replicates).  
944 The microcosms were randomly arranged in a growth chamber and grown for a 97-day growing  
945 period under controlled climatic conditions, as shown in Table 1.

### 946 *2.3 Soil sampling and analysis*

947 Soil samples were collected at three sampling times: at the beginning (pre-sowing), at pea  
948 flowering (62 days after sowing (DAS)) and at the pods filling pea stage (97 DAS), when the  
949 microcosms were destructively sampled for soil and plant collection. Each microcosm provided one  
950 rhizosphere sample (two samplings) and one bulk soil sample (three samplings). The rhizosphere  
951 soil was taken from the plant roots after the bulk of the soil had been removed. The rhizosphere soil  
952 was used for the molecular analysis and the bulk soil was used for the chemical and biochemical



953 characterization.

954 Soil chemical properties were determined according to standards methods recommended by the  
955 Soil Science Society of America (Sparks, 1996). Dissolved organic carbon (DOC) was extracted  
956 with water (1:2 w/v, soil:water) after shaking (170 rpm, 30 min) at room temperature. The soil  
957 slurries were then centrifuged (4300 rpm, 10°C, 10 min) and the recovered supernatant was filtered  
958 through a 0.45 µm Whatman GF/F membrane. DOC in the clean extract was finally measured using  
959 an automated elemental OC analyzer (Rosemount-Dohrmann DC-80) (Jones *et al.*, 2005) using a  
960 perchlorate oxidation followed by detection of CO<sub>2</sub> by NIR spectroscopy. Inorganic-N (NO<sub>3</sub><sup>-</sup>-N and  
961 NH<sub>4</sub><sup>+</sup>-N) was extracted with 1 M KCl (1:5, w/v, soil:solution) after shaking (220 rpm, 60 min) at  
962 24°C. After the extraction, the soil slurries were centrifuged (4300 rpm, 10 min) and the clean  
963 supernatants recovered and stored at -20°C before analysis. Inorganic N was determined using a  
964 continuous flow auto-analyser (SKALAR San<sup>++</sup>, BV, NL).

965 Microbial biomass C (MBC) and N (MBN) were determined following a chloroform  
966 fumigation-extraction (CFE) procedure according to Vance *et al.* (1987) and Brookes *et al.* (1985).  
967 MBC was estimated using a conversion factor of  $K_{EC} = 0.45$  (Joergensen, 1996) and MBN was  
968 estimated using a conversion factor of  $K_{EN} = 0.54$  (Joergensen and Mueller, 1996). Soil basal  
969 respiration was estimated by measuring CO<sub>2</sub> emissions in sealed 1.5 L jars containing 20 g (dw  
970 equivalent) soil samples and incubated in the dark at 24 °C. Gas samples were collected in pre-  
971 evacuated 22 ml vials and analysed by gas chromatography (Sparling, 1981). The cumulative CO<sub>2</sub>-  
972 C evolved after a 28-day incubation period (gas sampling was carried out after 1, 4, 7, 14, 21 and 28  
973 days) was assumed as R<sub>bas</sub>. The potentially mineralisable C (C<sub>0</sub>) was estimated by fitting the 28-day  
974 cumulative data to the first-order exponential function  $C_t = C_0 (1 - e^{-kt})$  (Riffaldi *et al.*, 1996). The best  
975 fitting of the equation to the values experimentally obtained and estimates of C<sub>0</sub> and k parameters  
976 for each curve of basal respiration were obtained by non-linear regression analysis using the  
977 Levenburg-Marquardt algorithm (Table Curve 2D v 5.01 software, SYSTAT software Inc.).  
978 Potentially mineralisable N (PMN), resulting from net mineralization of active soil organic N

979 occurring during the 28-day incubation period for  $R_{bas}$  determination, was estimated as the  
980 cumulative inorganic soil N after 28 days *minus* the inorganic soil N at 0 day (Drinkwater *et al.*,  
981 1996). The following soil eco-physiological indices were then calculated: the microbial quotient  
982 ( $MBC/C_{org}$ ), the metabolic quotient ( $qCO_2$ ), the mineralization coefficient ( $qM=R_{bas}/C_{org}$ ) and the  
983  $qCO_2/C_{org}$  ratio (Dilly *et al.*, 2001; Mocali *et al.*, 2009).

984 DNA extraction from both rhizosphere and bulk soil were undertaken by ball milling samples to  
985 achieve physical lysis followed by a CTAB-buffer extraction method as described by Brierley *et al.*  
986 (2009). DNA extracts were purified from any humic acids by passing them through micro Bio-spin  
987 columns loaded with polyvinylpyrrolidone (PVP). DNA yield and quality were quantified by a  
988 spectrophotometer (ND-1000). Automated ribosomal intergenic spacer analysis (ARISA) was  
989 carried with an end-point PCR technique using the primer system 1406f (5'-  
990 TGYACACACCGCCCGT-3') and 23Sr (5'-GGGTTBCCCCATTCRG-3'). The PCR reaction  
991 mixture was prepared with GoTaq<sup>®</sup> Green Master Mix (Promega), 2  $\mu$ l of template DNA (ca 20 ng),  
992 0.5  $\mu$ M of each primer, and sterile deionised water to a final volume of 25  $\mu$ l. In the negative  
993 control, the tDNA was substituted with the same volume of nuclease-free water (Promega). PCR  
994 running conditions started with a single denaturation step of 94 °C for 3 min, to activate the  
995 HotStart enzyme, followed by 29 thermal cycles consisting of a denaturation step at 94 °C for 45 s,  
996 an annealing step at 55 °C for 1 min, and an elongation step at 72 °C for 2 min, followed by a final  
997 primer extension at 72 °C for 7 min and cooling to 4 °C. Capillary electrophoresis with peaks  
998 ranging from 50-bp to 1,050-bp was carried out using an DNA 7500 assays on the Agilent 2100  
999 Bioanalyzer (Analysis Software 2100, Agilent Technologies, Böblingen, D) according to  
1000 manufacturer instructions. Electropherograms were imported into BioNumerics<sup>®</sup> 7.0 software  
1001 package (AppliedMaths, Sint-Martens-Latem, B) as a 2D gel image for further analysis.

1002

#### 1003 *2.4 Greenhouse gas monitoring*

1004 Emissions of  $N_2O$ , carbon dioxide ( $CO_2$ ) and methane ( $CH_4$ ) from the microcosm units were

1005 measured following three gas sampling strategies: soil surface emissions, deep layer emissions (23  
1006 cm) and respiration from roots. Surface gas monitoring started 12 days after sowing and was  
1007 repeated (twice a week) across the entire experimental period by using the closed chamber  
1008 technique (Smith *et al.*, 1995). During the gas emission measurements, the microcosms were  
1009 covered by a 26-cm-tall chamber for 40-60 minutes before collecting 40 ml gas samples in a  
1010 portable pre-evacuated 22-ml-glass vial (Scott *et al.*, 1999). For baseline corrections two air  
1011 samples from the growing chamber atmosphere were collected at each sampling time. Gas sampling  
1012 from deep soil layers started 38 days after sowing (14<sup>th</sup> November 2011) to allow time for the roots  
1013 to grow throughout the microcosm and was repeated twice a week for three weeks. Gaseous  
1014 emissions from legume roots collected after the microcosm destructive sampling (see below) were  
1015 measured as described by Inaba *et al.* (2009). Shortly after the harvest, unwashed legume roots were  
1016 placed into a 320 ml air-tight glass jars; 0 and 10 min after sealing, a 40-ml-gas sample was  
1017 collected from the glass jar and immediately transferred in a pre-evacuated 22 ml glass vial. All gas  
1018 samples were stored (maximum 1 day) in a controlled temperature room before any analysis.  
1019 Amounts of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> of collected air samples were analyzed using an Agilent 6890 gas  
1020 chromatograph equipped with a 1.8 m Propak-N column and an electron capture detector (for N<sub>2</sub>O)  
1021 and flame ionisation detector (for CH<sub>4</sub>). Certified high purity gas standards of known concentration  
1022 were used for calibration. The conversion of peak areas to daily gaseous emissions was carried out  
1023 in accordance with standard procedures (de Kleine *and* Harvey, 2013). In addition, greenhouse gas  
1024 emission intensities were expressed per unit of product (all emissions divided by all saleable  
1025 outputs. Also the Global Warming Potential (GWP) of each gas was calculated using coefficients of  
1026 1 for CO<sub>2</sub>, 25 for CH<sub>4</sub> and 298 for N<sub>2</sub>O.

## 1027 *2.5 Plant sampling and analysis*

1028 At pea flowering (62 DAS) and pod filling (97 DAS), the microcosms were destructively  
1029 sampled, plants were gently removed from the microcosm soil and separated into shoot and root  
1030 fractions. Shoot fresh weight was immediately recorded, whereas the root system was initially used

1031 for measuring the N<sub>2</sub>O emissions (legumes only). The above ground biomass results were used for  
1032 the emission intensities calculations.

## 1033 2.6 Statistics

1034 Soil variables were firstly checked for deviations from normality (Shapiro Wilk's test) and  
1035 homogeneity of within-group variances (Levene's test). The block effect in the experimental design  
1036 was not significant ( $P > 0.05$ ) and the data were subjected to the following statistical analyses. A  
1037 three-way analysis of variance (ANOVA) (treatment (T) x amendment (A) x time (Ti)), indicated in  
1038 Figs. 1, 2 and 4 and in Table 2 as  $F$ -values and corresponding  $P$ -values, was performed in order to  
1039 highlight the main effect of sampling time, crops, level of amendment and their interactions on  
1040 measured soil variables. Significant effects due to treatment (T), amendment (A), and their  
1041 interaction presented in Tab. 4 were estimated by a two-way ANOVA. Multiple pairwise  
1042 comparison of means were assessed by Tukey's HSD (Honestly Significant Difference) test at  $P <$   
1043  $0.05$  level of significance. Chemical and biochemical data were also analysed by principal  
1044 component analysis (PCA) with no rotation with data from three different stages (pre-sowing,  
1045 flowering and harvest) (Table 3 and 4). Statistical analyses were run using the Systat 11.0 software  
1046 (SYSTAT Software Inc., Erkrath, D). Graphs were drawn by using the SigmaPlot 10.0 software  
1047 (SYSTAT Software Inc.). Dendrograms of hierarchical classification of ARISA profiles were  
1048 generated by cluster analysis using the unweighed pair-group method with arithmetic averages  
1049 (UPGMA) based on Dice similarity coefficient as suggested by Rademaker *et al.* (1999).

## 1050 3. Results

### 1051 3.1 Soil C pools

1052 Soil carbon pools showed variable responses to the addition of plant residues and the presence of  
1053 different crop cultivars during the experiment. The addition of wheat residues in the microcosm  
1054 soils caused some significant reductions in the amount of the total organic carbon (C<sub>org</sub>) (Table 2),  
1055 although residue incorporation affected the C<sub>org</sub> differently in treatments over time. In particular, in

1056 unamended soils,  $C_{\text{org}}$  values remained close to the initial values; whereas following wheat residue  
1057 addition, a contrasting affect was observed in  $C_{\text{org}}$  content between monocropped treatments were  
1058 found to have the highest  $C_{\text{org}}$  concentrations. In bare soil  $C_{\text{org}}$  slightly declined, whereas it  
1059 remained practically unaffected in amended ones.

1060 Dissolved organic carbon (DOC) varied in response to residue addition levels and sampling  
1061 stages (Fig. 1). The presence of the intercrops increased the concentrations of DOC at harvest.  
1062 Without residues addition, no significant difference was observed between treatments at any  
1063 sampling stage; whereas following wheat residue addition the Zero4 treatment showed a significant  
1064 increase ( $P < 0.001$ ) from pre-sowing ( $36.9 \mu\text{g g}^{-1}$ ) to harvest ( $64.1 \mu\text{g g}^{-1}$ ). On average, DOC  
1065 increased over time from an initial value of 33.2 (or 37.3) to 48.2 (or 50.5)  $\mu\text{g g}^{-1}$  in unamended (or  
1066 amended) microcosms soil, including the bare soil which showed an increasing trend over time.

1067 In unamended microcosms, mean soil basal respiration,  $R_{\text{bas}}$ , values were higher than pre-  
1068 sowing at both flowering and harvest stage (respectively 778.9 and harvest 807.4  $\mu\text{g CO}_2\text{-C g}^{-1} 28 \text{ d}^{-1}$ )  
1069 <sup>1</sup>) and there was no significant effect due to the crop treatment (Fig. 1). However, residue  
1070 amendment strongly influenced ( $P < 0.05$ ) the  $\text{CO}_2$  emission of treatments at the harvest stage,  
1071 which ranged between 553.5 (bare soil) and 1042.6  $\mu\text{g CO}_2\text{-C g}^{-1} 28 \text{ d}^{-1}$  (Nitouche monocropping):  
1072 the Nitouche solo crop showed higher basal respiration than those at beginning of the experiment  
1073 (from 721.7 to 1042.6  $\mu\text{g CO}_2\text{-C g}^{-1} 28 \text{ d}^{-1}$ ), whereas in the bare soil  $R_{\text{bas}}$  decreased by  
1074 approximately 20% (from 664.6 to 553.5  $\mu\text{g CO}_2\text{-C g}^{-1} 28 \text{ d}^{-1}$ ). Estimates of the potentially  
1075 mineralisable carbon ( $C_0$ ) followed the same general trend as those of  $R_{\text{bas}}$ , even though some of the  
1076 experimental factors lost their significance (Fig. 1). It is noteworthy that  $C_0$  displayed a time-  
1077 dependent fluctuation with particularly high C mineralization from Triticale (differing from  $R_{\text{bas}}$ )  
1078 and Nitouche monocrops.

1079 Microbial biomass carbon (MBC) was strongly affected by treatments with statistically  
1080 significant responses to all the experimental factors (Fig. 1). In general the MBC increased during  
1081 the cropping season, in spite of residue amendment: from an initial 79.0 (or 75.5) to final 191.4 (or

1082 243.6)  $\mu\text{g C g}^{-1}$  in unamended (or amended) soil microcosms. In soils with no wheat residue  
1083 addition, MBC showed a large increase in the presence of legume-based treatments either in  
1084 monocropped - from mean initial 79.1 to final 209.3  $\mu\text{g C g}^{-1}$  (approximately +265%) - or  
1085 intercropped legumes - from initial 75.5 to final 233.8  $\mu\text{g C g}^{-1}$  (approximately +310%). An  
1086 opposite affect was observed in residue amended soils: the MBC increase was generally lower  
1087 under intercropping (+290%) than in monocropping (+390%) as compared with the starting value of  
1088 75.7  $\mu\text{g C g}^{-1}$ .

### 1089 3.2 Soil N pools

1090 Time and time x amendment were the only factors that significantly affected the variability of  
1091 total nitrogen content ( $N_t$ ) in microcosms soils (Table 2). In fact,  $N_t$  content decreased across the 97-  
1092 day experimental period with differing trends, but reaching similar values at the harvest stage (2.09  
1093 and 2.01  $\text{g kg}^{-1}$  for unamended and amended, respectively) (data not shown). Across the  
1094 experimental period, the extractable  $\text{NH}_4^+$ -N did not differ significantly in any of the treatments  
1095 (Fig. 2); however, the amount of soil nitrate showed marked time-dependent fluctuations and was  
1096 significantly different among treatments ( $P < 0.001$ ). Crop growth markedly affected the dynamics  
1097 of soil nitrate-N, which became greatly depleted at the flowering stage in all planted microcosms.  
1098 An increased release of nitrate was observed at the latest stage, also mirrored by a decline in the  
1099 ammonium-N content, yet regulated by the decaying wheat residues (Fig. 2).

1100 The potentially mineralisable nitrogen (PMN) was affected by all the experimental factors and  
1101 their interactions (Fig. 2). In general, PMN demonstrated a clear decrease from pre-sowing onward.  
1102 At the flowering stage, PMN in the bare soil treatment was significantly higher ( $P < 0.01$ ) than the  
1103 treatments with no residue addition. It was noteworthy that, at the harvest stage, PMN was  
1104 significantly affected by residue amendment, even though at a different level ( $P < 0.05$  and  $P <$   
1105  $0.001$ , respectively). Specifically, Nitouche monocropping increased the PMN by three times from  
1106 the flowering stage reaching the highest value of 13.4  $\mu\text{g g}^{-1} 28\text{d}^{-1}$  in microcosms packed without  
1107 addition of wheat residues. All the remaining cropping treatments showed a small non-significant

1108 increase, but the bare soil retained similar values ( $10.81 \mu\text{g g}^{-1} 28 \text{ d}^{-1}$ ). Further, in amended soils,  
1109 there was a significant increase in the Triticale - Zero4 intercropping from the flowering ( $5.8 \mu\text{g g}^{-1}$   
1110  $28 \text{ d}^{-1}$ ) to the harvest stage ( $12.3 \mu\text{g g}^{-1} 28 \text{ d}^{-1}$ ).

1111 All experimental factors and their interactions statistically influenced the microbial biomass N  
1112 ( $P < 0.001$ ). In unamended microcosms, MBN moderately (intercrops) or strongly (monocrops)  
1113 increased over time, with the exception of the bare soil treatment where it decreased from the  
1114 beginning of the experimental period ( $14.6 \mu\text{g N g}^{-1}$ ) by approx. 40% (from 14.6 to  $8.8 \mu\text{g N g}^{-1}$ ). In  
1115 contrast, in residue amended soils, the unplanted soil showed MBN values statistically comparable  
1116 to other cropping treatments: as a whole MBN increased by approx. 70%, from initial 17.0 to final  
1117  $28.8 \mu\text{g N g}^{-1}$  (Fig. 2).

### 1118 *3.3 Soil ecophysiological indices and C-to-N ratios*

1119 The mineralization coefficient ( $qM$ ) was statistically influenced by the amendment level ( $P <$   
1120  $0.001$ ), time ( $P < 0.001$ ) and their interactions (Fig. 3). In unamended soils, the mineralization  
1121 coefficient values showed a slight increase, on average from  $16.79 \mu\text{g CO}_2\text{-C mg}^{-1} \text{C}_{\text{org}}$  (pre-sowing  
1122 stage) to  $24.35 \mu\text{g CO}_2\text{-C mg}^{-1} \text{C}_{\text{org}}$  (harvest sampling). In microcosms added with wheat residues,  
1123 it showed an opposite trend for Zero4, Triticale and bare soil, which showed the major decline  
1124 (from  $25.14 \mu\text{g CO}_2\text{-C mg}^{-1} \text{C}_{\text{org}}$  to  $18.80 \mu\text{g CO}_2\text{-C mg}^{-1} \text{C}_{\text{org}}$ ).

1125 The metabolic quotient ( $q\text{CO}_2$ ) was significantly ( $P < 0.001$ ) affected only by time, level of soil  
1126 amendment and their interaction (Fig. 3). Microcosms at both level of amendment showed a  
1127 decrease in the values of the  $q\text{CO}_2$  towards the end of the experiment, which was stronger in the  
1128 amended soil due to the higher average values it showed in the pre-sowing stage (1.11 and  $2.57 \mu\text{g}$   
1129  $\text{CO}_2\text{-C mg}^{-1} \text{MBC d}^{-1}$  respectively for unamended and amended). The largest decrease was  
1130 registered in the Nitouche pure culture (from 2.69 to  $0.19 \mu\text{g CO}_2\text{-C mg}^{-1} \text{MBC d}^{-1}$ ). The  $q\text{CO}_2/\text{C}_{\text{org}}$   
1131 ratio was also statistically influenced by time ( $P < 0.001$ ), level of soil amendment ( $P < 0.001$ ) and  
1132 their interactions (Fig. 3). However, in amended microcosms, the  $q\text{CO}_2/\text{C}_{\text{org}}$  ratio clearly decreased  
1133 in all treatments from pre-sowing to harvest stage.

1134 The microbial quotient ( $MBC/C_{org}$ ) was strongly ( $P < 0.001$ ) affected by all the experimental  
1135 factors (Fig. 3).  $MBC/C_{org}$  varied consistently during the experimental period and showed a marked  
1136 increase at the harvest stage in all treatments at both amendment levels. The bare soil treatment  
1137 always showed the lowest value within each sampling time, reaching its minimum at the flowering  
1138 stage in residue amended microcosms ( $2.09 \mu\text{g MBC mg}^{-1} C_{org}$ ).

### 1139 *3.4 Soil pH and electrical conductivity*

1140 The three-way ANOVA revealed that wheat residue addition was the main factor affecting the  
1141 variability of pH data ( $P < 0.001$ ), which were generally higher in the amended soil (Table 2). There  
1142 were also a time-dependent fluctuations ( $P < 0.01$ ) together with significant effects of the  
1143 amendment x time, and amendment x time x treatment interactions ( $P < 0.001$ ). However, the pH  
1144 varied between a narrow range comprised between 6.12 (unamended bare soil at flowering) and  
1145 6.37 (amended triticale at flowering), and significant differences among treatments were only  
1146 noticed at the flowering and the harvest stages in the unamended soil with the Nitouche monocrop  
1147 and bare soil having, respectively, the highest (6.39) and the lowest value (6.10).

1148 The electrical conductivity ( $EC_{1:2}$ ) varied between 0.10 and 0.18  $\text{dS m}^{-1}$  and was significantly  
1149 affected by most of the experimental factors and their interactions (Table 2). It was noticeable that  
1150 the triticale-based treatments showed higher  $EC_{1:2}$  values than the leguminous sole treatments at  
1151 both flowering and harvest stages: this finding was only observed in unamended, but not in the  
1152 amended microcosms, and this was especially true for all crop-based treatments where EC remained  
1153 almost constant over time. In the bare soil, the lowest EC was found in unamended treatments  
1154 ( $\sim 0.10 \text{ dS m}^{-1}$ ); whereas following wheat residues addition it increased considerably at flowering  
1155 and harvest stage, respectively to 0.18 and 0.15  $\text{dS m}^{-1}$ .

### 1156 *3.5 Multivariate analysis*

1157 According to the eigenvalue  $> 1.0$  criterion only five principal components could be selected. The  
1158 first two principal components PC1 (eigenvalue 5.37) and PC2 (eigenvalue 2.79) explained a large



1159 portion (33.55 and 17.44%, respectively) of the total variance. The following three components PC3  
1160 (eigenvalue 2.17), PC4 (eigenvalue 1.35) and PC5 (eigenvalue 1.06) accounted for 13.54, 8.43 and  
1161 6.63% of total variance, respectively. Since the first two components taken together explained as  
1162 much as 50.98% of the total variance, we focused on them (Table 3). Firstly, it is worth noting that  
1163 PC1 was primarily weighed by either C-related functional variables (DOC,  $qM$ ,  $R_{bas}$ , MBC and  
1164  $MBC/C_{org}$ ) or N-related variables (PMN and  $N_t$ ). It was also found that PC2 was primarily affected  
1165 by one of the most dynamic N pools in soil:  $NH_4^+$ -N, which was also directly related to  $qCO_2$  and  
1166  $qCO_2/C_{org}$ . On the other hand MBN was the only variable affecting PC3. Moreover, PC4 was  
1167 weighed by  $C_{org}$  and pH. Whereas, in PC5 PMC was the only soil variable showing a loading factor  
1168 close to the reference threshold value (0.60). In the ordination biplot of Factor 1 vs Factor 2, soil  
1169 samples from the differing treatments appeared in most cases well separated at least in three main  
1170 groups along the PC axis 1 (functional C variables and N-related properties): triticale  
1171 monocropping, Nitouche - Triticale intercropping and, surprisingly, a rather broad group including  
1172 all the other crop treatments plus the bare soil. On the other hand, the two leguminous monocrops  
1173 were clearly separated along the PC axis 2 (Fig. 4A).

1174 The two first principal components PC1 (eigenvalue 5.21) and PC2 (eigenvalue 2.92) expressed a  
1175 somewhat large portion (32.59 and 18.22%, respectively) of the total variance. The following three  
1176 components PC3 (eigenvalue 2.04), PC4 (eigenvalue 1.46) and PC5 (eigenvalue 1.22) accounted  
1177 for 12.77, 9.14 and 7.60% of total variance, respectively. Once again, we focused on the first two  
1178 PCs as they explained as much as half of the variance (50.81%) (Table 4). PC1 was primarily  
1179 weighed by either C-related functional variables (MBC,  $MBC/C_{org}$ ,  $qCO_2/C_{org}$ ,  $qCO_2$  and DOC) or  
1180 N-related variables (MBN, PMN and  $N_t$ ). PC2 was primarily affected by some C-related functional  
1181 variables ( $R_{bas}$ ,  $qM$  and  $C_0$ ) and  $NO_3^-$ -N.  $C_{org}$  was the only variable affecting PC3. EC was the only  
1182 variable affecting PC4.. In the ordination biplot of Factor 1 vs Factor 2, soil samples from the  
1183 amended microcosms were rather scattered onto the plot: the two intercropping combinations were  
1184 closely associated, whereas the two leguminous monocrops were not. The bare soil was well

1185 separated from the other treatments. Noticeably, among the chemical and biochemical soil  
1186 variables,  $\text{NO}_3^-$ -N and C ( $\text{C}_0$ ) exerted a primary role in separating the treatments along the PC2 axis  
1187 (Fig. 4B).

### 1188 3.6 ARISA analysis

1189 The molecular structure of the bacterial communities profiles were characterized by the number  
1190 and length distribution of major bands which, in spite of treatments and residue levels, were  
1191 observed in a fragment size range from 200 to 1000 bp, and showed a clear diversity between levels  
1192 of residue. In particular, regardless of the growth stage, residues addition in soils appeared to  
1193 enhance the difference in groups allowing the monocropped legumes and Triticale-based treatment  
1194 to clearly group on separate clusters (~78%; Fig. 5). On the contrary, in the no-residue soils, the  
1195 treatment-dependent communities did not clearly align on the endemic axis, not allowing the  
1196 clusters to present a clear pattern. The only clear difference was between bare soil and other  
1197 treatments, which showed a level of similarity of approximately 73%.

### 1198 3.7 Greenhouse gases (GHGs) emissions

1199 Nitrous oxide emissions from the amended treatments were lower in comparison to the unamended  
1200 soils ( $P < 0.001$ ). In the amended treatments, the emissions started to pick up after 60 days of the  
1201 start of the experiment with the unplanted treatment having the highest emissions (81.25 g  $\text{N}_2\text{O}$ -N  
1202  $\text{ha}^{-1} \text{ day}^{-1}$ ). In the unamended treatments, the emissions were higher ( $P < 0.05$ ) in the Triticale  
1203 monocrop and Triticale/Nitouche treatments including also the no plant treatment from 30 days  
1204 after the seeding.

1205 The cumulative values of  $\text{N}_2\text{O}$  were higher in the unamended treatments at 82 days. The bare soil  
1206 treatment had the highest emissions in both treatments (4319 and 1430 g  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$  in unamended  
1207 and amended, respectively). In the microcosms with crop, the Triticale/Nitouche treatment had the  
1208 highest (3677 g  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$ ) and the Triticale/Zero 4 the lowest (356 g  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$ ) emissions in  
1209 unamended soils ( $P < 0.001$ ). In the amended treatments, the cumulative emissions were generally

1210 very low, and even showed consumption of N<sub>2</sub>O (negative values) with similar patterns in the  
1211 unamended soils (Triticale/Nitouche: 243 g N<sub>2</sub>O-N ha<sup>-1</sup> and Triticale/Zero4: -550 g N<sub>2</sub>O-N ha<sup>-1</sup>)  
1212 (Table 5). Below ground N<sub>2</sub>O emissions showed a similar pattern between amendment levels during  
1213 the experimental period. However, the concentration of N<sub>2</sub>O was ten times greater from the no  
1214 residue treatment in comparison with the residue ( $P < 0.001$ ). The bare soil treatment had the  
1215 highest average values (19.70 ppm and 1.95 ppm for the no residue and residue, respectively)  
1216 followed by the Triticale/Nitouche treatment (2.56 and 1.30 ppm for the unamended and amended,  
1217 respectively) (Table 5).

1218 Cumulative CO<sub>2</sub> emissions were highest in the Zero 4 treatment (2511 kg CO<sub>2</sub>-C ha<sup>-1</sup>) in  
1219 unamended soils and the Nitouche (2790 kg CO<sub>2</sub>-C ha<sup>-1</sup>) under residue addition (Table 5). The bare  
1220 soil treatment had the highest average belowground concentration of CO<sub>2</sub> in both residue treatments  
1221 during the experimental period ( $P < 0.001$ ) (Table 5). Methane emissions were low during the  
1222 experimental period for both level of amendment without (Table 5).

1223 Emission intensities presented in this paper include the cumulative N<sub>2</sub>O measurements (84 out of 97  
1224 days) for the total biomass produced within this time providing an index of the effectiveness of  
1225 mitigation. In the residue treatment, the Triticale/Zero4 had the lowest emission intensities of all the  
1226 treatments (-393 g N<sub>2</sub>O t biomass<sup>-1</sup>). N<sub>2</sub>O intensities were not significant different for the no residue  
1227 treatment (Table 6).

## 1228 **4. Discussion**

1229 The results obtained from this study provide new insights into the interrelated effects of  
1230 leguminous crops on the chemical and biochemical properties of soil and highlights the important  
1231 differences in C and N cycling associated with pea-based intercropping and wheat residue  
1232 incorporation.

### 1233 *4.1 Soil chemical properties*

1234 Even in simplified ecosystems such as microcosms, soil organic carbon can be considered one of

1235 the most important indicators of soil quality because of its important role in the maintenance of soil  
1236 structure, microorganisms and nutrient cycling (Aalders *et al.*, 2009).

1237 Soil incorporation of wheat residues slightly reduced the total organic carbon ( $C_{org}$ ), which  
1238 appeared noticeable in the ANOVA analysis but resulted negligible impacts in the principal  
1239 component analysis either with or without residue addition. Indeed, it could be anticipated that total  
1240 soil organic matter, would not respond rapidly to environmental changes, unless major amendments  
1241 are made (Powelson *et al.*, 1987). However, mixing occurring during the establishment of the  
1242 experimental units was expected to alter soil C dynamic and enhance rates of soil organic matter  
1243 degradation, thus leading to a so-called tillage effect (Linsler *et al.*, 2013; Tortorella and Gelsomino,  
1244 2011). The observation that soils receiving residue inputs were associated with lower organic C and  
1245 N pools would indicate that the addition of residues had stimulated the microbial populations and  
1246 increased the decomposition of preexisting organic matter through a priming effect (Kuzyakov  
1247 2002). This increased degradation activity not only influenced the carbon but also the nitrogen  
1248 cycling, which is functionally interconnected in soil, and thus resulted in a more striking variation  
1249 in  $N_t$  than in the  $C_{org}$ .

1250 Even if major changes in total organic carbon content may be difficult to detect over a short-term  
1251 experiment (Haynes, 1999), the responses of more labile fractions of soil organic carbon, namely  
1252 dissolved organic carbon (DOC), are much more sensitive to soil management than total soil  
1253 organic matter (Silveira, 2005). This fraction markedly influences soil chemical, biological and  
1254 physical properties, as a primary source of mineralizable C, N, P, and S (Haynes, 2000) and it has  
1255 been proposed as an indicator of the size of the available C pool to soil microorganisms (Boyer and  
1256 Groffman, 1996).

1257 Through their exudates, plant root systems represents a major source of C flow entering the soil  
1258 and stimulating the microbial process of immobilisation/release of soluble organic compounds  
1259 forming the DOC pool in soil (Paterson, 2003; Paterson *et al.*, 2007). In fact, the quality and  
1260 amount of rhizodeposition released from the legumes root systems could explain the high

1261 significance showed by the crop factor on the variability of this parameter in this study (Fustec *et*  
1262 *al.*, 2010).

1263 The addition of plant residues and fresh organic compounds through rhizodepositions most often  
1264 results in a net N immobilisation phase followed by a net re-mineralisation phase. In our study,  
1265 lower amounts of inorganic N were observed in the treatment with wheat residue addition than in  
1266 the corresponding unamended treatment. Wheat residue incorporation seems to have enhanced net  
1267 N immobilization, although N mineralization was promoted in presence of the legume treatment at  
1268 the end of the incubation period.

1269 The significant difference between amendment levels and crop presence shown suggests a  
1270 different effect of faunal activity on residues. This could be due to increased available N in soil,  
1271 which is consequently is not limiting for soil microorganisms responsible for degrading the  
1272 residues. However, Knapp *et al.* (1983) reported conflicting evidence where some studies found  
1273 mixed results from the effect of N availability on residue decomposition.

1274 Soil pH was fairly resilient to changes during the microcosm experiment (as clearly shown by  
1275 PCA analysis) and this was actually not unexpected since it is not a highly variable parameter, and  
1276 is often resilient also to short term perturbations (Table 3 and 4).

#### 1277 *4.2 Soil biochemical properties*

1278 This study confirms, as previously suggested (Ndiaye *et al.*, 2000), that biological and  
1279 biochemical parameters are more sensitive and can provide earlier measurements of changes  
1280 produced by different soil and crop management than physical and chemical indicators. Most  
1281 authors have studied the quantity and the activity of soil microbial biomass as indicator of changes  
1282 driven by the addition of organic residue or cropping systems (Kaiser and Heinemeyer, 1993;  
1283 Ndiaye *et al.*, 2000).

1284 Microbial biomass, is known to be one of the main drivers of nutrient cycling in soils, with  
1285 microbial activity releasing essential nutrients to plants and microbial biomass is functionally and  
1286 closely linked to the turnover of soil organic carbon (Jenkinson and Ladd, 1981). It is therefore of

1287 significance that the soil microbial biomass showed a greater increase, in all legume based  
1288 treatments in the unamended soil. This increase, observed at the last sampling, could have been due  
1289 to higher growth of microbial biomass, induced by the legume crop (Dinesh *et al.*, 2004). The  
1290 statistically significant difference shown in the microbial biomass dynamics in response to the  
1291 presence/absence of residues can depend on the decomposition rate of plant material and on the  
1292 microbial immobilisation processes. In fact, the N assimilation requirements are determined by this  
1293 carbon flow (Mary *et al.*, 1996). It is often assumed that N coming from the residue and from  
1294 recycled biomass is mineralised before being assimilated by the newly-formed biomass. However, it  
1295 has been shown that the soil microflora can directly assimilate significant amounts of organic N  
1296 compounds coming from plant residues or from decaying biomass.

1297 Furthermore, the introduction of the residue amendment increased soil basal respiration as  
1298 measured by cumulative CO<sub>2</sub> emissions. Although R<sub>bas</sub> was not responsive to the individual  
1299 treatments, it was markedly influenced by the interactions they determined with the amendment.  
1300 This finding can suggest that in this soil the metabolic activity was primarily influenced by  
1301 compositional changes in soil organic matter due an enhanced residue decomposition of the organic  
1302 compounds released from plants roots.

#### 1303 *4.3 Analysis of soil microbial community structures*

1304 The results obtained in this study confirm that the addition to the soil of crop residues can  
1305 strongly modify the genetic structure of the community by stimulating particular populations;  
1306 especially as the soil system is often substrate-limited as regards microbial growth (Nicolardot *et*  
1307 *al.*, 2007). In fact, the molecular analysis revealed that the genetic structures of the bacterial  
1308 population itself were significantly changed in response to the presence of legume sole crops or  
1309 triticale, either in association with the legume or in monocropping, as a function of the  
1310 presence/absence of wheat residue in the soils.

#### 1311 *4.4 Greenhouse Gas emissions*

1312 Our study demonstrated that there were lower N<sub>2</sub>O emissions from legumes, which is consistent  
1313 with our understanding that there are low levels of N<sub>2</sub>O emission associated with the fixation  
1314 process (Rochette *and* Janzen 2005). The results are also consistent with those of Pappa *et al* (2011)  
1315 showing higher emissions from the pea cultivar Nitouche both as a monocrop and when grown as  
1316 an intercrop. The Nitouche monocrop had up to six time higher emissions (434 g ha<sup>-1</sup>) than the  
1317 monocrop Zero 4 (71 g ha<sup>-1</sup>) in the amended treatment and twice in the unamended (749 and 374 g  
1318 ha<sup>-1</sup> for Nitouche and Zero 4, respectively). However, there was no significant difference between  
1319 the intercropping treatments. Intriguingly these higher emissions were observed in the absence of  
1320 wheat residue additions, and did not appear to be associated with elevated concentrations of DOC.  
1321 The denitrification processes driven by the availability of oxidisable carbon, which is used as a  
1322 terminal electron acceptor in the respiratory process. Therefore, the absence of higher levels of  
1323 DOC in the legumes was elevated emissions of N<sub>2</sub>O raises the possibility that the carbon was being  
1324 supplied by the legume itself. Support for this hypothesis would be provided by higher soil  
1325 respiration rates measured from Nitouche, even in the absence of acid plant residues and as  
1326 indicated in differences in microbial activity shown by the ARISA analysis. There is also a growing  
1327 body of evidence indicating that differences in rhizodeposition associated with different crop  
1328 cultivars may drive differences in N<sub>2</sub>O emissions (Gogoi & Baruah 2012; Sey *et al.* 2010)

1329 Plant species and combinations of species offer significant opportunities to modify soil derived  
1330 N<sub>2</sub>O emissions. If differential rates of rhizodeposition are able to alter denitrification rates, then  
1331 selecting specific legume cultivars with low rates of deposition in combination with cereals may  
1332 therefore provide a novel opportunity for the mitigation of N<sub>2</sub>O emissions. It is possible that the  
1333 mechanisms underlying these differences would be associated either with an improved capacity of  
1334 certain legume cultivars to compete more efficiently for soil N. Alternatively there may be an  
1335 interaction between the legume and soil microbial community that reduces N<sub>2</sub>O emission (possibly  
1336 by promoting increased rates of N immobilization). The choice of legume cultivar and species is  
1337 therefore a key factor influencing the amount of N loss. A previous study (Pappa *et al.*, 2011) has

1338 shown that the cultivar Zero 4 has significant lower N loss by N<sub>2</sub>O emissions and leaching and  
1339 could therefore contribute to the development of agricultural systems with environmental benefits.  
1340 Therefore having a better understanding of the varietal differences in selecting intercrops mixtures  
1341 has a high potential to increase yields and contribute towards the developments of agricultural  
1342 systems with environmental benefits.

## 1343 **5. Conclusions**

1344 Legumes are generally associated with lower emissions of N<sub>2</sub>O than cereal crops. However,  
1345 there is significant variability in emissions between different legume cultivars. In this study the  
1346 higher emissions associated with Nitouche were generated in the absence of wheat residues, raising  
1347 the possibility that this variation in emissions is driven by variations in carbon supplied from the  
1348 legume root. The intercrop affect on microbial activity is also cultivar specific. This is indicated by  
1349 differences in N<sub>2</sub>O emissions observed from two pea cultivars when grown as intercrops, although  
1350 differences in N<sub>2</sub>O emission were not linked to differences in yield. The mechanism underlying  
1351 these differences appears to be driven by the differences resulting from microbial activity, which in  
1352 turn are likely to be linked to soil-plant carbon dynamics.

1353 Our research therefore highlights the importance of the cultivar choice in the sustainable  
1354 agricultural systems. The addition of the residues affects the soil C pools and the N<sub>2</sub>O emissions and  
1355 shows clear differences between the two pea cultivars but also the intercropping combinations. The  
1356 root development of pea monocrops was influenced by the residue addition but also the presence of  
1357 cereal highlighting the complexity of such systems. The scale of these effects is highly sensitive to  
1358 management and soil type. The growing need for environmental tests of the legume cultivars to  
1359 understand further the mechanisms of the GHGs emissions is in high priority. Understanding the  
1360 development of legume cultivar and the interactions taking place within legume/cereal intercrop has  
1361 the potential to be a very useful management tool in the development of more sustainable  
1362 agricultural systems and in mitigation of GHG from agriculture.



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1374

1375 **References**

- 1376 Aalders, I., Hough, R.L., Towers, W., Black, H.I.J., Ball, B.C., Griffiths, B.S., Hopkins, D.W., Lilly,  
1377 A., McKenzie, B.M., Rees, R.M., 2009. Considerations for Scottish soil monitoring in the  
1378 European context. *Eur. J. Soil Sci.* 60, 833-843.
- 1379 Baggs, E.M., Watson, C.A., Rees, R.M., 2000. The fate of nitrogen from incorporated cover crop  
1380 and green manure residues. *Nutr. Cycl. Agroecosys.* 56, 153-163.
- 1381 Boyer, J.N., Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in  
1382 forest and agricultural soil profiles. *Soil Biol. Biochem.* 28, 783-790.
- 1383 Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002. Emissions of N<sub>2</sub>O and NO from fertilized  
1384 fields: Summary of available measurement data. *Global Biogeochem. Cycles* 16(4), 1058.
- 1385 Brierley, J.L., Stewart, J.A., Lees, A.K., 2009. Quantifying potato pathogen DNA in soil. *Appl. Soil*  
1386 *Ecol.* 41, 234-238.
- 1387 Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the  
1388 release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen  
1389 in soil. *Soil Biol. Biochem.* 17, 837-842.
- 1390 Corre-Hellou, G., Dibet, A., Hauggaard-Nielsen, H., Crozat., Y., Gooding, M., Ambus, P.,  
1391 Dahlmann, C., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2011. The competitive  
1392 ability of pea-barley intercrops against weeds and the interactions with crop productivity and  
1393 soil N availability. *Field Crop Res.* 122, 264-272.
- 1394 de Kleine, C., Harvey, M., 2013. Nitrous oxide chamber guidelines; Global Research Alliance on  
1395 Agricultural Greenhouse Gases. Ministry of Primary Industries, 25 The Terrace, PO Box 2526,  
1396 Wellington, New Zealand.
- 1397 Dilly, O., Winter, K., Lang, A., Munch, J.C., 2001. Energetic eco-physiology of the soil microbiota  
1398 in two landscapes of southern and northern Germany. *J. Plant Nutr. Soil Sci.* 164, 407-413.
- 1399 Dinesh, R., Suryanarayana, M.A., Ghosha, S., Sheeja, T.E., 2004. Long-term influence of  
1400 leguminous cover crops on the biochemical properties of a sandy clay loam Fluventic

1401 Sulfaquent in a humid tropical region of India. *Soil Till. Res.* 77, 69-77.

1402 Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralisable  
1403 nitrogen as an indicator of biologically active soil nitrogen. In: Doran J.W., Jones A.J. (Eds.),  
1404 Methods for Assessing Soil Quality. Soil Science Society of America, Special Publication 49,  
1405 Madison, WI. 217-229.

1406 FAO, 2001. Factors regulating nitrous oxide and nitric oxide emission. Available at  
1407 <http://www.fao.org/DOCREP/004/Y2780E/y2780e02.htm>

1408 Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.B., 2010. Nitrogen rhizodeposition of legumes. A  
1409 review. *Agron. Sustain. Dev.* 30, 57-66.

1410 Gogoi, B. & Baruah, K.K. 2012. Nitrous oxide emissions from fields with different wheat and rice  
1411 varieties. *Pedosphere*, 22, 112-121.

1412 Haynes, R.J., 1999. Labile organic matter fractions and aggregate stability under short-term grass-  
1413 based leys. *Soil Biol. Biochem.* 31, 1821-1830.

1414 Haynes, R.J., 2000. Labile organic matter as an indicator of organic matter quality in arable and  
1415 pastoral soils in New Zealand. *Soil Biol. Biochem.* 32, 211-219.

1416 Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2003. The comparison of nitrogen use and leaching  
1417 in sole cropped versus intercropped pea and barley. *Nutri. Cycl. Agroecosys.* 65, 289-300.

1418 Hauggaard-Nielsen, H., Jensen, E.S., 2005. Facilitative root interactions in intercrops. *Plant Soil*  
1419 274, 237-250.

1420 Hauggaard-Nielsen, H., Andersen, M.K., Jørnsgaard, B., Jensen, E.S., 2006. Density and relative  
1421 frequency effects on competitive interactions and resource use in pea-barley intercrops. *Field*  
1422 *Crop Res.* 95, 256-267.

1423 Hauggaard-Nielsen, H., Mundus, S., Jensen, E.S., 2009a. Nitrogen dynamics following grain  
1424 legumes and subsequent catch crops and the effect on succeeding cereal crops. *Nutri. Cycl.*  
1425 *Agroecosys.* 84, 281-291

1426 Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C.,

1427 Dibet, A., Von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009b. Pea-barley  
1428 intercropping for efficient symbiotic N<sub>2</sub>-fixation, soil N acquisition and use of other nutrients in  
1429 European organic cropping systems. *Field Crops Res.* 113, 64-71.

1430 Ikeda, S., Rallos, L.E.R., Ohkubo, T., Eda, S., Inaba, S., Mitsui, H., 2008. Microbial community  
1431 analysis of field-grown soybeans with different nodulation phenotypes. *Appl. Environ.*  
1432 *Microbiol.* 74, 5704–5709.

1433 Inaba, S., Tanabe, K., Eda, S., Ikeda, S., Higashitani, A., Mitsui, H., Minamisawa, K., 2009. Nitrous  
1434 oxide emission and microbial community in the rhizosphere of nodulated soybeans during the  
1435 late growth period. *Microbes Environ.* 24 (1), 64-67.

1436 Inal, A., Gunes, A., Zhang, F., Cacmak, I., 2007. Peanut/maize intercropping induced changes in  
1437 rhizosphere and nutrient concentrations in shoots. *Plant Physiol. Biochem.* 45, 350-356.

1438 Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: Measurement and turnover. In: *Soil*  
1439 *Biochemistry* vol 5 (Paul E.A., Ladd J.N., eds). Marcel Dekker, New York. pp. 415-471.

1440 Jensen, E.S., Peoples, M.B., Hauggaard-Nielsen, H., 2010. Faba bean in cropping systems. *Field*  
1441 *Crops Res.* 115, 203-216.

1442 Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass:  
1443 calibration of the K<sub>EC</sub> value. *Soil Biol. Biochem.* 28, 25-31.

1444 Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial  
1445 biomass: calibration of the K<sub>EN</sub> value. *Soil Biol. Biochem.* 28, 33-37.

1446 Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., Hodge, A., 2005. Dissolved organic nitrogen  
1447 uptake by plants. An important N uptake pathway? *Soil Biol. Biochem.* 37(3), 413-423.

1448 Kaiser, E.A., Heinemeyer, O., 1993. Seasonal variations of soil microbial biomass carbon within the  
1449 plow layer. *Soil Biol. Biochem.* 25, 1649-1655.

1450 Knapp, E.B., Elliott, L.F., Campbell, G.S., 1983. Carbon, nitrogen and biomass interrelationships  
1451 during the decomposition of wheat straw: a mechanistic simulation model. *Soil Biol. Biochem.*  
1452 15, 455-461.

- 1453 Knudsen, M.T., Hauggaard-Nielsen, H., Jørnsgaard, B., Jensen, E.S., 2004. Comparison of  
1454 interspecific competition and N use in pea-barley, faba bean-barley and lupin-barley intercrops  
1455 grown at two temperate locations. *J. Agric. Sci.* 142, 617-627.
- 1456 Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant*  
1457 *Nutrition and Soil Science-Zeitschrift für Pflanzenernährung und Bodenkunde*, 165, 382-396.
- 1458 Liebman, M., Dyck, E., 1993. Crop rotation and intercropping strategies for weed management.  
1459 *Ecol. Applic.* 3, 92-122.
- 1460 Linsler, D., Geisseler, D., Loges, R., Taube, F., Ludwig, B., 2013. Temporal dynamics of soil organic  
1461 matter composition and aggregate distribution in permanent grassland after a single tillage event  
1462 in a temperate climate. *Soil Till. Res.* 126, 90-99.
- 1463 Mary, B., Recous, S., Darwis, D., Robin, D., 1996. Interactions between decomposition of plant  
1464 residues and nitrogen cycling in soil. *Plant Soil.* 181, 71-82.
- 1465 Mocali, S., Dentice, A., Marcucci, A., Benedetti, A., 2009. The impact of post-harvest treatments of  
1466 transgenic eggplant residues on soil quality and microbial diversity. *Agrochimica.* 53(5), 296-  
1467 307.
- 1468 Ndiaye, E.L., Sandeno, J.M., Mcgrath, D., Dick, R.P., 2000. Integrative biological indicators for  
1469 detecting change in soil quality. *Am. J. Altern. Agric.* 15, 26-36.
- 1470 Nicolardot, B., Bouziri, L., Bastian, F., Ranjard, L., 2007. Influence of location and quality of plant  
1471 residues on residue decomposition and genetic structure of soil microbial communities. *Soil*  
1472 *Biol. Biochem.* 39, 1631-1644.
- 1473 O'Hara, G.W., Daniel, R.M., 1985. Rhizobial denitrification: a review. *Soil Biol. Biochem.* 17, 1-9.
- 1474 Okubo, T., Ikeda, S., Kaneko, T., Eda, S., Mitsui, H., Sato, S., 2009. Nodulation-dependent  
1475 communities of culturable bacterial endophytes from stems of field-grown soybeans. *Microbes*  
1476 *Environment.* 24, 253-258.
- 1477 Palmqvist, K., Dahlman, L., 2006. Responses of the green algal foliose lichen *Platismatia glauca* to  
1478 increased nitrogen supply. *New Phytol.* 171(2), 343-56.

- 1479 Pappa, V.A., Rees, R.M., Walker, R.L., Baddeley, J.A., Watson, C.A., 2011. Intercropping reduces  
1480 nitrous oxide emissions and leaching from an arable rotation. *Agric. Ecosyst. Environ.* 141(1-2),  
1481 153-161.
- 1482 Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial  
1483 productivity. *Eur. J. Soil Sci.* 54, 741-750.
- 1484 Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere  
1485 microbial community structure in organic soil. *New Phytol.* 173, 600-610.
- 1486 Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S., Germon, J.C., 2002. Molecular analysis of the  
1487 nitrate-reducing community from unplanted and maize-planted soils. *Appl. Environ. Microbiol.*  
1488 68, 6121-6128.
- 1489 Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass  
1490 provides an early indication of changes in total soil organic-matter due to straw incorporation.  
1491 *Soil Biol. Biochem.* 19, 159-164.
- 1492 Rademaker, J.L.W., Louws, F.J., Rossbach, U., Vinuesa, P., De Bruijn, F. J., 1999. Computer-  
1493 assisted pattern analysis of molecular fingerprints and database construction. In: Akkermans A.  
1494 D. L., van Elsas J. D., de Bruijn F. J. (Eds.), *Molecular Microbial Ecology Manual*, 1–33 sect.  
1495 7.1.3. Kluwer Academic Publishers, Dordrecht, NL.
- 1496 Riffaldi, R., Saviozzi, A., Levi-Minzi, R., 1996. Carbon mineralization kinetics as influenced by  
1497 soil properties. *Biol. Fertil. Soils.* 22, 293-298.
- 1498 Rochette, P., Janzen, H., 2005. Towards a revised coefficient for estimating N<sub>2</sub>O emissions from  
1499 legumes. *Nutr. Cycl. Agroecosys.* 73, 171-179.
- 1500 Scalise, A., Tortorella, D., Pristeri, A., Petrovičová, B., Gelsomino, A., Lindström, K., Monti, M.,  
1501 2015. Legume-barley intercropping stimulates soil N supply and crop yield in the succeeding  
1502 durum wheat in a rotation under rainfed conditions. *Soil Biol. Biochem.* 89, 150-161.
- 1503 Scott, A., Chrichton, I., Ball, B.C., 1999. Long-term monitoring of soil gas emissions with closed  
1504 chambers using automated and manual systems. *J. Environ. Qual.* 28, 1637-1643.

1505 Sey, B.K., Manceur, A.M., Whalen, J.K., Gregorich, E.G. & Rochette, P. 2010. Root-derived  
1506 respiration and nitrous oxide production as affected by crop phenology and nitrogen  
1507 fertilization. *Plant and Soil*, 326, 369-379.

1508 Silveira, M.L.A., 2005. Dissolved organic carbon and bioavailability of N and P as indicators of soil  
1509 quality. *Sci. Agric.* 62, 502-508.

1510 Smith, K.A., Clayton, H., McTaggart, I.P., Arah, J.R.M., Scott, A., 1995. The measurements of  
1511 nitrous oxide from soil using chambers. *Philos. Trans. R. Soc. London, Series B.* 351, 327-338.

1512 Sparling, G.P., 1981. Microcalorimetry and other methods to assess biomass and activity in soil.  
1513 *Soil Biol. Biochem.* 13(2), 93-98.

1514 Sparks, D.L., 1996. *Methods of Soil Analysis, Part 3 - Chemical Methods.* SSSA Book Series No. 5.  
1515 SSSA-ASA, Madison, WI.

1516 Tortorella D., Gelsomino A., 2011. Influence of compost amendment and maize root system on soil  
1517 CO<sub>2</sub> efflux: a mesocosm approach. *Agrochimica.* 3, 161-177.

1518 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil  
1519 microbial biomass C. *Soil Biol. Biochem.* 19, 703-707.

1520 Watson, C.A., Baddeley, J.A., Edwards, A.C., Rees, R.M., Walker, R.L., Topp, C.F.E., 2011.  
1521 Influence of ley duration on the yield and quality of the subsequent cereal crop (spring oats) in  
1522 an organically managed long-term crop rotation experiment. *Org. Agric.* 1, 147-159.

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1528 **Table 1** – Chamber growth conditions during the 97-day experimental period were in accordance to  
1529 the 26-year average climatic data recorded between April and August in a Mediterranean  
1530 environment (Reggio Calabria, Southern Italy). The relative humidity was kept stable at 70%.  
1531 Lighting was produced by cool white fluorescent bulbs at an average intensity of 1160 lux.

<b>Growth period (day)</b>	<b>Temperature (°C)</b>		<b>Photoperiod (h) Day/Night</b>
	<b>Day</b>	<b>Night</b>	
<b>0-20</b>	18.2 ± 0.3	10.2 ± 0.3	6.5/17.5
<b>21-40</b>	23.4 ± 0.3	14.6 ± 0.2	8/16
<b>41-60</b>	28.0 ± 0.3	18.9 ± 0.3	9.5/14.5
<b>61-80</b>	30.6 ± 0.3	21.7 ± 0.2	10.5/13.5
<b>81-97</b>	31.2 ± 0.4	22.5 ± 0.2	9.5/14.5

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1534 **Table 2** – Soil pH, EC, organic C and total N in the soil (mean  $\pm$  SD,  $n = 3$ ) was measured at the  
 1535 beginning and at the end of the experimental period. Symbols – and + represent absence or presence  
 1536 of amendment in soils. For each sampling time, different letters in the columns indicate significant  
 1537 differences among treatments (Tukey’s HSD test at  $P < 0.05$ ). Significant effects due to treatment,  
 1538 amendment, time and their interactions on the variability of data ( $F$ -value from three-way ANOVA,  
 1539 treatment x amendment x time, with corresponding  $P$  values<sup>a</sup>) are also shown at the bottom.

		Treatment	pH	EC <sub>1:2</sub> (dS m <sup>-1</sup> )	C <sub>org</sub> (mg g <sup>-1</sup> )	N <sub>t</sub> (mg g <sup>-1</sup> )	
Pre-sowing	Nitouche	-	6.19 $\pm$ 0.06	0.10 $\pm$ 0.01	34.13 $\pm$ 1.75	2.50 $\pm$ 0.10	
		+	6.24 $\pm$ 0.07	0.11 $\pm$ 0.01	29.94 $\pm$ 5.34	2.33 $\pm$ 0.29	
	Zero4	-	6.17 $\pm$ 0.04	0.11 $\pm$ 0.01	34.13 $\pm$ 1.75	2.50 $\pm$ 0.10	
		+	6.29 $\pm$ 0.03	0.11 $\pm$ 0.01	28.97 $\pm$ 4.41	2.33 $\pm$ 0.28	
	Triticale	-	6.21 $\pm$ 0.04	0.10 $\pm$ 0.01	34.87 $\pm$ 0.92	2.53 $\pm$ 0.06	
		+	6.23 $\pm$ 0.06	0.11 $\pm$ 0.01	30.07 $\pm$ 5.48	2.50 $\pm$ 0.20	
	Triticale/Nitouche	-	6.18 $\pm$ 0.04	0.10 $\pm$ 0.01	34.40 $\pm$ 0.26	2.53 $\pm$ 0.06	
		+	6.26 $\pm$ 0.08	0.11 $\pm$ 0.01	33.73 $\pm$ 1.60	2.60 $\pm$ 0.10	
	Triticale/Zero4	-	6.19 $\pm$ 0.06	0.10 $\pm$ 0.01	33.83 $\pm$ 1.24	2.50 $\pm$ 0.10	
		+	6.24 $\pm$ 0.07	0.11 $\pm$ 0.01	33.47 $\pm$ 2.06	2.60 $\pm$ 0.11	
	Bare soil	-	6.21 $\pm$ 0.04	0.11 $\pm$ 0.01	34.27 $\pm$ 1.76	2.53 $\pm$ 0.12	
		+	6.28 $\pm$ 0.03	0.11 $\pm$ 0.01	29.97 $\pm$ 5.58	2.50 $\pm$ 0.10	
	Harvest	Nitouche	-	6.31 $\pm$ 0.04 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>b,c</sup>	36.36 $\pm$ 4.56	2.15 $\pm$ 0.02
			+	6.25 $\pm$ 0.03	0.12 $\pm$ 0.01 <sup>a,b</sup>	35.03 $\pm$ 7.51	2.12 $\pm$ 0.15
Zero4		-	6.24 $\pm$ 0.04 <sup>a,b</sup>	0.10 $\pm$ 0.01 <sup>c</sup>	32.19 $\pm$ 0.92	2.06 $\pm$ 0.03	
		+	6.27 $\pm$ 0.04	0.12 $\pm$ 0.02 <sup>b</sup>	37.54 $\pm$ 3.51	2.07 $\pm$ 0.01	
Triticale		-	6.18 $\pm$ 0.03 <sup>a,b</sup>	0.13 $\pm$ 0.01 <sup>a</sup>	30.32 $\pm$ 1.37	2.08 $\pm$ 0.06	
		+	6.31 $\pm$ 0.03	0.10 $\pm$ 0.01 <sup>b</sup>	33.62 $\pm$ 2.31	2.05 $\pm$ 0.06	
Triticale/Nitouche		-	6.21 $\pm$ 0.03 <sup>a,b</sup>	0.12 $\pm$ 0.01 <sup>a,b</sup>	36.96 $\pm$ 1.52	2.09 $\pm$ 0.10	
		+	6.30 $\pm$ 0.03	0.11 $\pm$ 0.01 <sup>b</sup>	28.12 $\pm$ 2.12	1.87 $\pm$ 0.09	
Triticale/Zero4		-	6.21 $\pm$ 0.02 <sup>a,b</sup>	0.12 $\pm$ 0.01 <sup>a,b</sup>	34.62 $\pm$ 1.06	2.10 $\pm$ 0.01	
		+	6.28 $\pm$ 0.07	0.10 $\pm$ 0.01 <sup>b</sup>	30.58 $\pm$ 2.05	1.96 $\pm$ 0.19	

Bare soil	-	6.16 ± 0.01 <sup>b</sup>	0.10 ± 0.01 <sup>c</sup>	30.98 ± 0.43	2.09 ± 0.07
	+	6.29 ± 0.05	0.15 ± 0.01 <sup>a</sup>	28.89 ± 1.30	1.98 ± 0.32
<b>Factor</b>	<b>df</b>				
Treatment (T)	5	1.866 <sup>ns</sup>	1.866 <sup>ns</sup>	1.172 <sup>ns</sup>	0.510 <sup>ns</sup>
Amendment (A)	1	67.903 <sup>***</sup>	67.903 <sup>***</sup>	5.815 <sup>*</sup>	2.276 <sup>ns</sup>
Time (Ti)	2	5.026 <sup>**</sup>	5.026 <sup>**</sup>	2.238 <sup>ns</sup>	121.046 <sup>***</sup>
T x A	5	8.130 <sup>***</sup>	8.130 <sup>***</sup>	0.558 <sup>ns</sup>	0.146 <sup>ns</sup>
T x Ti	10	1.350 <sup>ns</sup>	1.350 <sup>ns</sup>	1.571 <sup>ns</sup>	1.204 <sup>ns</sup>
Ti x A	2	0.500 <sup>ns</sup>	0.500 <sup>ns</sup>	1.982 <sup>ns</sup>	16.213 <sup>***</sup>
T x A x Ti	10	3.024 <sup>**</sup>	3.024 <sup>**</sup>	2.897 <sup>**</sup>	1.051 <sup>ns</sup>
Error	72				

1540 <sup>a</sup> Levels of significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns: not significant.

1541 **Table 3** – Soil Principal component analysis (PCA) of 16 soil chemical and biochemical variables  
1542 measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4  
1543 - Triticale, Bare soil as in Materials and Methods) in the unamended soils during the 97-day  
1544 microcosm experiment. PC loading variables (values  $\geq |0.60|$  are in bold) and percent of total  
1545 variance explained by the first five factors (eigenvalue  $>1$ ) are reported. Soil variables are as  
1546 described in Materials and Methods.

Soil variable	PC1	PC2	PC3	PC4	PC5
PMN	<b>-0.82</b>	-0.32	-0.11	-0.06	-0.32
N <sub>t</sub>	<b>-0.82</b>	-0.24	0.14	0.01	-0.13
R <sub>bas</sub>	<b>0.82</b>	0.15	-0.39	-0.18	-0.23
MBC/C <sub>org</sub>	<b>0.80</b>	-0.35	0.27	0.23	0.14
MBC	<b>0.79</b>	-0.34	0.34	0.11	0.14
qM	<b>0.75</b>	0.07	-0.54	0.11	-0.15
DOC	<b>0.73</b>	0.34	0.28	0.07	0.11
C <sub>0</sub>	0.58	-0.07	-0.34	-0.23	<b>-0.60</b>
NH <sub>4</sub> <sup>+</sup> -N	-0.01	<b>0.83</b>	0.06	-0.23	-0.19
qCO <sub>2</sub>	-0.24	<b>0.78</b>	-0.14	0.26	0.17
qCO <sub>2</sub> /C <sub>org</sub>	-0.20	<b>0.72</b>	-0.27	0.37	0.13
MBN	0.37	0.01	<b>0.70</b>	0.12	-0.17

<b>NO<sub>3</sub><sup>-</sup>-N</b>	0.40	-0.45	-0.50	0.10	0.25
<b>C<sub>org</sub></b>	-0.01	0.13	0.49	<b>-0.63</b>	0.03
<b>pH</b>	0.09	0.15	0.45	<b>0.60</b>	-0.52
<b>EC</b>	0.48	0.44	0.17	-0.37	0.12
<b><i>Variance explained (%)</i></b>	<b>33.55</b>	<b>17.44</b>	<b>13.54</b>	<b>8.43</b>	<b>6.63</b>

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1549 **Table 4** – Principal component analysis (PCA) of 16 soil chemical and biochemical variables  
 1550 measured in the six experimental treatments (Nitouche, Zero4, Triticale, Nitouche - Triticale, Zero4  
 1551 - Triticale, Bare soil as in Materials and Methods) in the amended soils during the 97-day  
 1552 microcosm experiment. PC loading variables (values  $\geq |0.60|$  are in bold) and percent of total  
 1553 variance explained by the first five factors (eigenvalue  $>1$ ) are reported. Soil variables are as  
 1554 described in Materials and Methods.

Soil variable	PC1	PC2	PC3	PC4	PC5
MBC	<b>0.92</b>	-0.19	-0.01	-0.04	0.25
MBC/ C <sub>org</sub>	<b>0.92</b>	-0.18	-0.18	-0.05	0.16
qCO <sub>2</sub> / C <sub>org</sub>	<b>-0.82</b>	0.15	0.01	0.18	0.20
qCO <sub>2</sub>	<b>-0.79</b>	0.17	0.16	0.22	0.23
MBN	<b>0.77</b>	-0.27	-0.17	0.18	0.30
N <sub>t</sub>	<b>-0.77</b>	-0.05	0.11	0.05	0.38
DOC	<b>0.61</b>	0.29	0.53	0.02	-0.07
PMN	<b>-0.61</b>	-0.28	-0.52	-0.26	0.26
R <sub>bas</sub>	0.36	<b>0.83</b>	-0.22	0.15	0.07
C <sub>0</sub>	0.09	<b>0.78</b>	-0.32	0.17	0.35
NO <sub>3</sub> <sup>-</sup> -N	-0.01	<b>-0.71</b>	-0.18	0.56	-0.13
qM	0.20	<b>0.66</b>	-0.59	0.06	-0.13
NH <sub>4</sub> <sup>+</sup> -N	-0.21	0.53	0.39	-0.20	-0.41
C <sub>org</sub>	0.18	0.17	<b>0.68</b>	0.19	0.56
EC	-0.01	0.06	0.03	<b>0.91</b>	-0.28
pH	0.13	-0.07	0.48	-0.06	-0.02
<i>Variance explained (%)</i>	<i>32.59</i>	<i>18.22</i>	<i>12.77</i>	<i>9.14</i>	<i>7.60</i>

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1556 **Table 5** – Average above ground flux emissions ( $\mu\text{g ml}^{-1}$ ) for the whole experimental period for  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{CH}_4$  followed by carbon dioxide  
 1557 equivalent, expressed in t, and then average below ground flux emissions ( $\mu\text{g ml}^{-1}$ ) for the whole experimental period for  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{CH}_4$ . Symbols  
 1558 – and + represent absence or presence of amendment in soils. Significant effects due to treatment, amendment and their interaction on the variability of  
 1559 soil data ( $F$ -values from two-way ANOVA, treatment x amendment, with corresponding  $P$  values<sup>b</sup>) are also shown at the bottom.

Treatment		Above ground			Below ground		
		$\text{N}_2\text{O}$	$\text{CO}_2$	$\text{CH}_4$	$\text{N}_2\text{O}$	$\text{CO}_2$	$\text{CH}_4$
Nitouche	-	$0.39 \pm 0.16^b$	$1620.34 \pm 1022.89$	$2.10 \pm 0.81$	$0.47 \pm 0.04^b$	$2805.17 \pm 639.15^b$	$2.34 \pm 0.13$
	+	$0.38 \pm 0.10$	$2253.45 \pm 1608.46$	$2.10 \pm 0.78$	$0.59 \pm 0.12^b$	$5075.28 \pm 701.74^b$	$2.17 \pm 0.11^b$
Zero4	-	$0.33 \pm 0.11^b$	$2076.57 \pm 1869.64$	$2.07 \pm 0.81$	$0.66 \pm 0.11^b$	$4386.84 \pm 719.72^b$	$2.00 \pm 0.14$
	+	$0.34 \pm 0.09$	$2219.74 \pm 1795.18$	$2.06 \pm 0.86$	$0.72 \pm 0.15^b$	$7073.73 \pm 992.48^b$	$2.05 \pm 0.14^b$
Triticale	-	$0.59 \pm 0.55^{a,b}$	$2077.48 \pm 1704.66$	$2.12 \pm 0.51$	$2.08 \pm 1.00^b$	$3048.84 \pm 447.91^b$	$2.12 \pm 0.15$
	+	$0.37 \pm 0.15$	$2156.48 \pm 1722.16$	$2.14 \pm 0.86$	$0.56 \pm 0.08^b$	$10373.01 \pm 1115.54^b$	$2.26 \pm 0.09^b$
Triticale/Nitouche	-	$0.86 \pm 0.38^a$	$1920.04 \pm 1616.19$	$2.35 \pm 0.67$	$2.56 \pm 1.17^b$	$7799.00 \pm 1167.89^b$	$2.10 \pm 0.14$
	+	$0.30 \pm 0.11$	$2243.43 \pm 1870.47$	$2.05 \pm 0.86$	$1.30 \pm 0.42^{a,b}$	$7714.24 \pm 1584.48^b$	$2.29 \pm 0.09^b$
Triticale/Zero4	-	$0.38 \pm 0.07^{a,b}$	$2054.57 \pm 1878.98$	$2.11 \pm 0.78$	$0.87 \pm 0.33^b$	$3725.17 \pm 415.45^b$	$2.23 \pm 0.12$
	+	$0.36 \pm 0.10$	$2388.89 \pm 2103.67$	$2.07 \pm 0.82$	$0.67 \pm 0.14^b$	$7743.60 \pm 683.45^b$	$2.18 \pm 0.12^b$
Bare soil	-	$0.80 \pm 0.33^{a,b}$	$1687.66 \pm 1205.27$	$2.17 \pm 0.73$	$19.70 \pm 4.76^a$	$7907.47 \pm 1193.37^a$	$2.31 \pm 0.16$
	+	$0.36 \pm 0.08$	$2389.69 \pm 2266.00$	$2.14 \pm 0.89$	$1.95 \pm 0.37^a$	$17480.05 \pm 1398.95^a$	$3.28 \pm 0.37^a$
<b>Factor</b>	<b>df</b>						
<b>Treatment (T)</b>	<b>5</b>	3.286 **	0.050 ns	0.062 ns	12.008 ***	14.550 ***	4.173 **
<b>Amendment (A)</b>	<b>1</b>	18.408 ***	1.064 ns	0.141 ns	13.033 **	44.209 ***	3.216 ns
<b>T x A</b>	<b>5</b>	4.116 **	0.083 ns	0.093 ns	9.221 ***	5.023 ***	2.374 *
<b>Error</b>	<b>84</b>						

1560 <sup>a</sup> Different letters in a column indicate significant differences among treatments (Tukey's test at  $P < 0.05$ ). <sup>b</sup> Levels of significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns: not significant.

1561 **Table 6** – Emission intensities (total cumulative N<sub>2</sub>O measurements divided by the total  
 1562 biomass for the whole experimental period), expressed in g per t of total biomass. Symbols –  
 1563 and + represent absence or presence of amendment in soils. Significant effects due to  
 1564 treatment, amendment and their interaction on the variability of soil data (*F*-values from two-  
 1565 way ANOVA, treatment x amendment, with corresponding *P* values<sup>b</sup>) are also shown.

Treatment		Intensities		
		N <sub>2</sub> O	CO <sub>2</sub>	CH <sub>4</sub>
Nitouche	-	0.04 ± 0.41	219.30 ± 302.67	171.22 ± 62.25
	+	0.01 ± 0.04	933.45 ± 258.87	143.11 ± 98.77
Zero4	-	0.92 ± 0.33	1066.66 ± 201.18	182.58 ± 153.10
	+	-0.02 ± 0.48	1119.81 ± 647.68	58.53 ± 175.01
Triticale	-	1.33 ± 0.84	898.48 ± 789.79	199.41 ± 310.07
	+	-0.11 ± 0.36	1159.32 ± 256.00	462.84 ± 691.76
Triticale/Nitouche	-	0.68 ± 1.15	774.83 ± 1465.21	166.11 ± 167.84
	+	0.24 ± 0.35	2104.40 ± 1861.08	543.02 ± 879.48
Triticale/Zero4	-	0.18 ± 0.40	846.15 ± 47.80	355.79 ± 44.58
	+	-0.30 ± 0.31	1492.26 ± 1035.41	180.94 ± 409.14
<b>Factor</b>	<b>df</b>			
<b>Treatment (T)</b>	<b>4</b>	1,754 <sup>ns</sup>	0,603 <sup>ns</sup>	0,566 <sup>ns</sup>
<b>Amendment (A)</b>	<b>1</b>	8,884 <sup>**</sup>	2,008 <sup>ns</sup>	0,098 <sup>ns</sup>
<b>T x A</b>	<b>4</b>	2,463 <sup>ns</sup>	0,219 <sup>ns</sup>	0,462 <sup>ns</sup>
<b>Error</b>	<b>18</b>			

1566 <sup>a</sup> Different letters in a column indicate significant differences among treatments (Tukey's test at *P* <  
 1567 0.05).

1568 <sup>b</sup> Levels of significance: \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; ns: not significant.

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1571 **Figure captions**

1572 **Fig. 1.** Changes in soil dissolved organic C (DOC), basal respiration ( $R_{\text{bas}}$ ), potential  
1573 mineralisable C ( $C_0$ ) and microbial biomass C (MBC) (mean  $\pm$  SD,  $n=3$ ) in unamended (left)  
1574 and amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-  
1575 day experimental period for the six treatments: Nitouche, Zero4, Triticale, Triticale-Nitouche,  
1576 Triticale-Zero4, bare soil.

1577 **Fig. 2.** Changes in KCl-extractable ammonium-N ( $\text{NH}_4^+$ -N), KCl-extractable nitrate-N ( $\text{NO}_3^-$ -  
1578 N), potential mineralisable N (PMN) and microbial biomass N (MBN) (mean  $\pm$  SD,  $n=3$ ) in  
1579 unamended (left) and amended (right) microcosm soils at three sampling times (0, 62 and 97  
1580 DAS) over the 97-day experimental period. Treatments are as in Fig. 1.

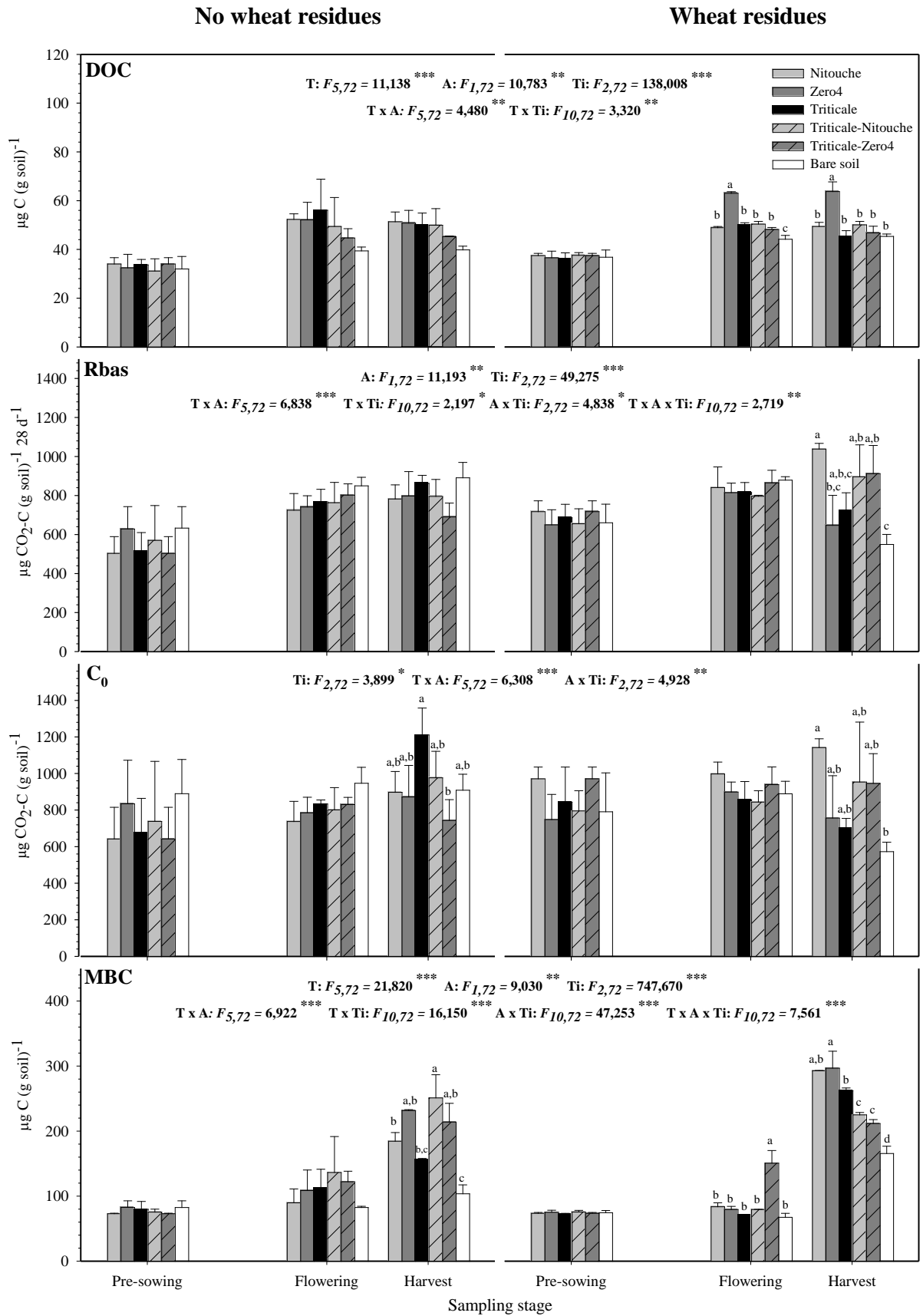
1581 **Fig. 3.** Changes in mineralization coefficient ( $qM$ ), metabolic quotient ( $q\text{CO}_2$ ),  $q\text{CO}_2/C_{\text{org}}$   
1582 ratio and microbial quotient ( $\text{MBC}/C_{\text{org}}$ ) (mean  $\pm$  SD,  $n=3$ ) in unamended (left) and  
1583 amended (right) microcosm soils at three sampling times (0, 62 and 97 DAS) over the 97-day  
1584 experimental period. Treatments are as in Fig. 1.

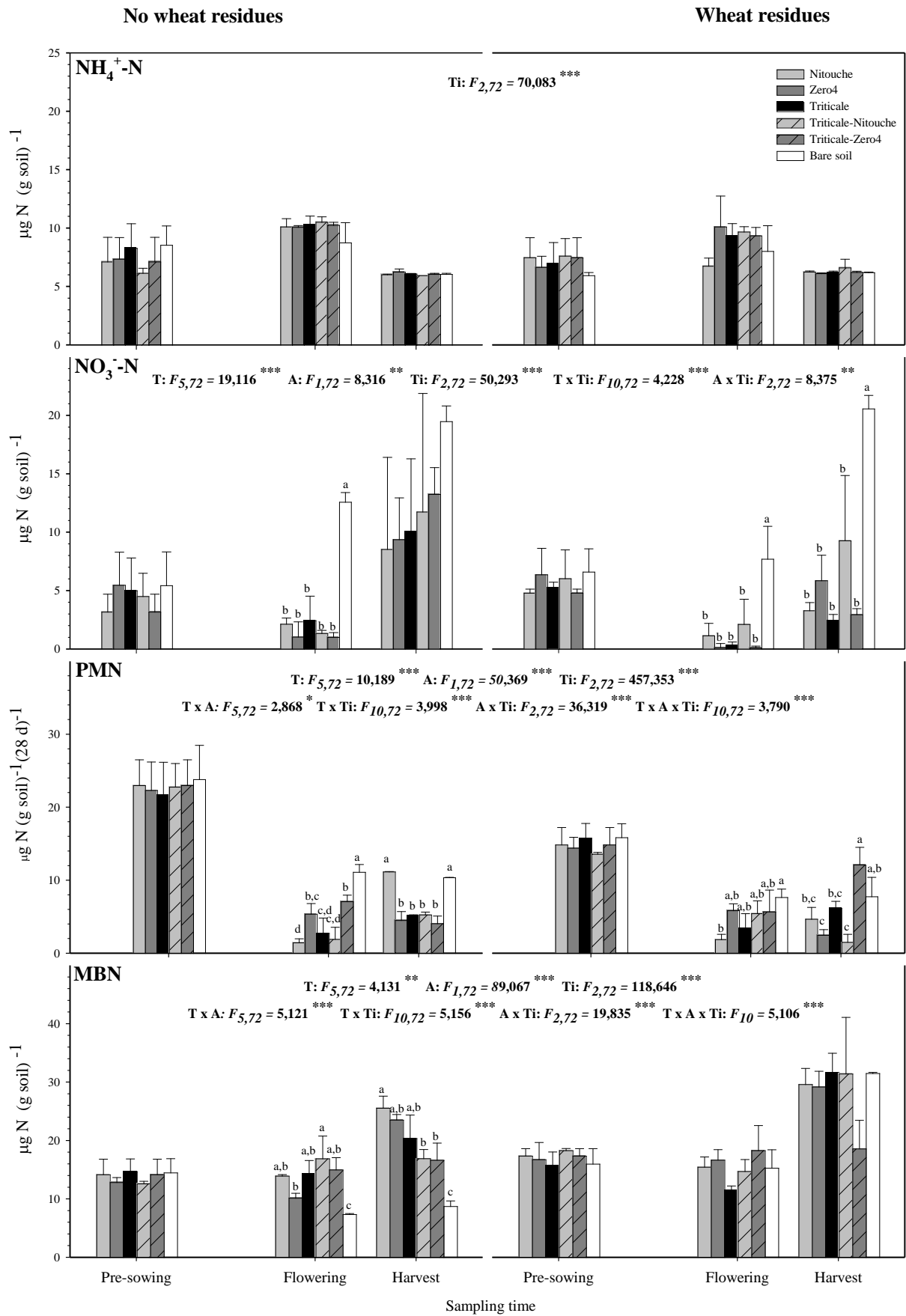
1585 **Fig. 4.** PCA ordination biplot (PC1 vs PC2) of 16 soil chemical and biochemical variables  
1586 (loadings, see Materials and Methods) measured in the six experimental treatments (Nitouche,  
1587 Zero4, Triticale, Triticale-Nitouche, Triticale-Zero4, bare soil as in Materials and Methods)  
1588 (scores) at three sampling times (pre-sowing, flowering, harvest) in the unamended (A) and  
1589 the amended soils (B) during the 97-day microcosm experiment. The biplot has the same  
1590 origin for scores and loadings.

1591 **Fig. 5.** Hierarchical classification (Pearson's similarity coefficient, Ward's clustering method)  
1592 of banding patterns generated by ARISA of PCR-amplified 16S rRNA gene-coding fragments  
1593 from soil-extracted bacterial DNA from no-residue (A) and residue (B) added microcosms at

1594 two sampling times (62 and 97 DAS) over the 97-day experimental period. Treatments are as  
1595 in Fig. 1. Each bar averages three microcosm replicates. Scale bar (0–100) indicates the  
1596 similarity level.  
1597

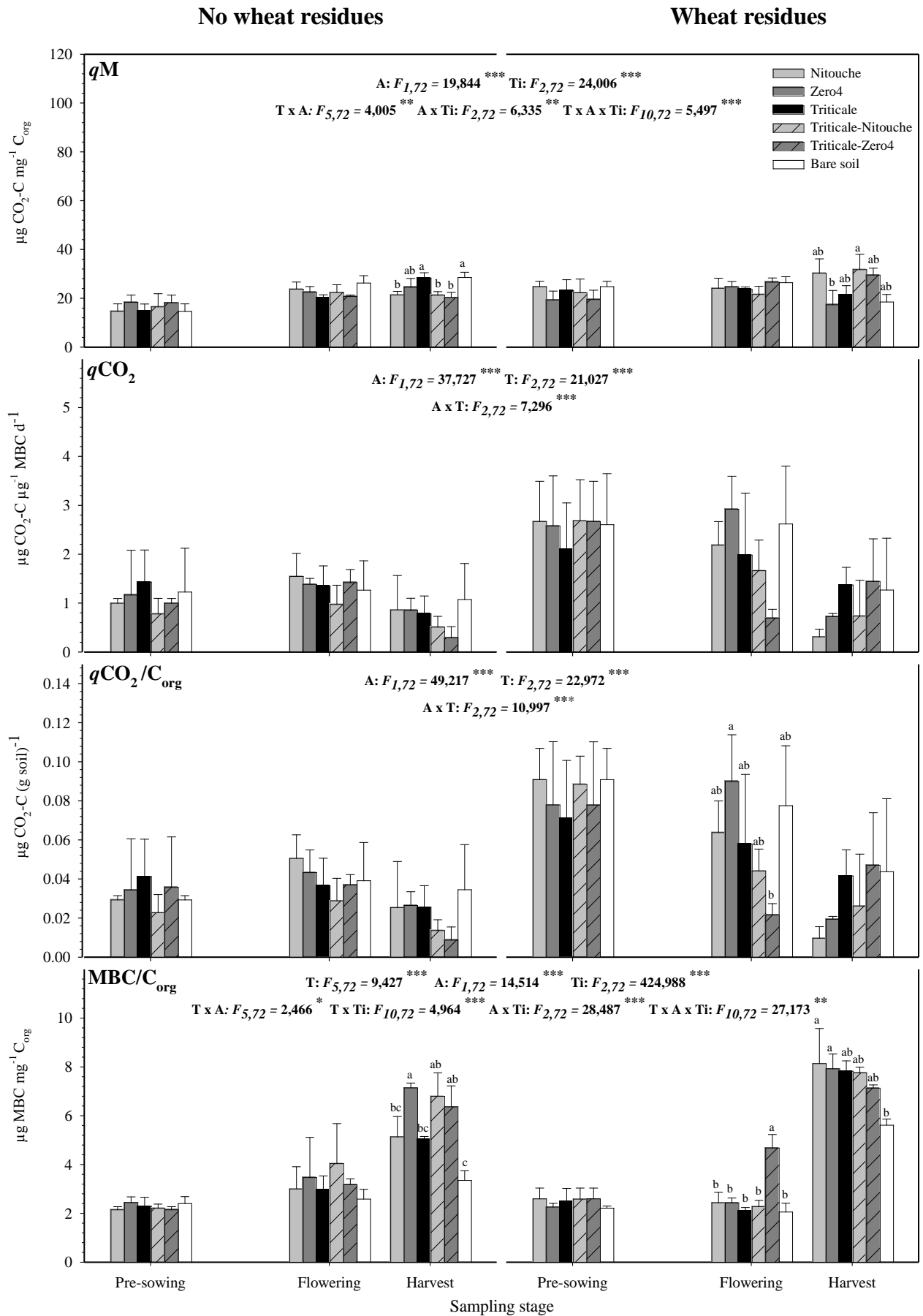




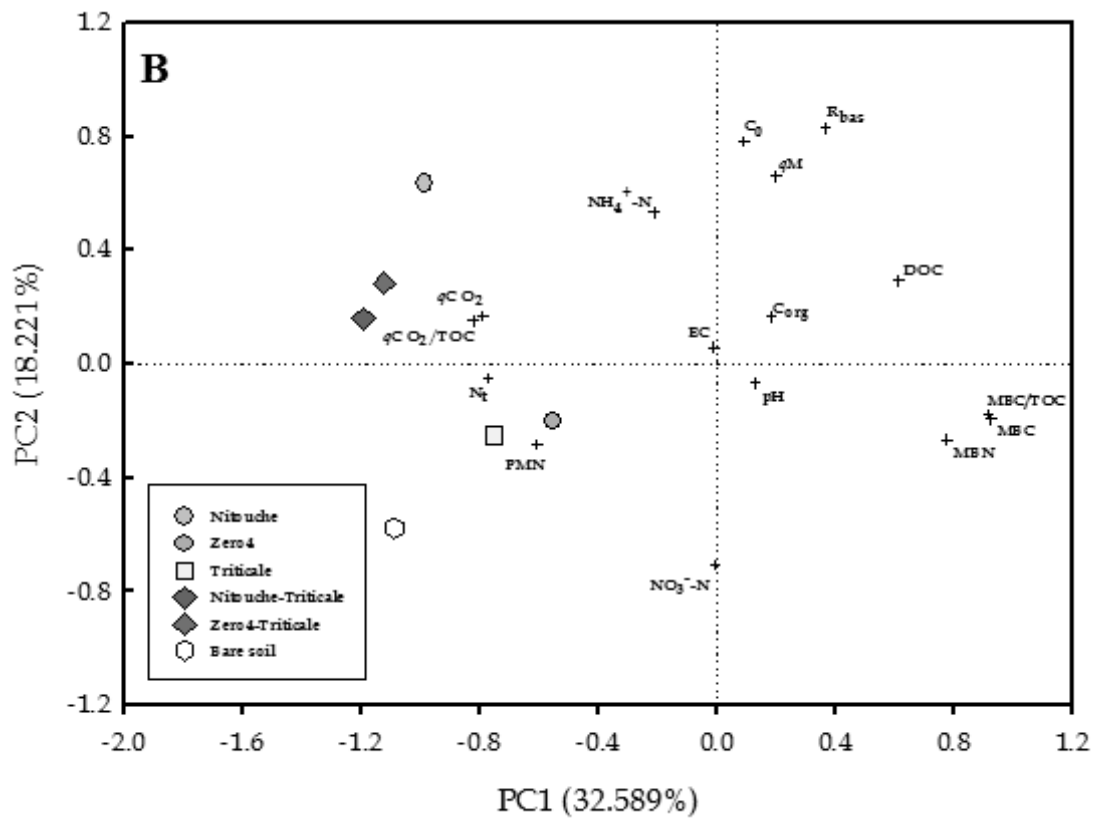
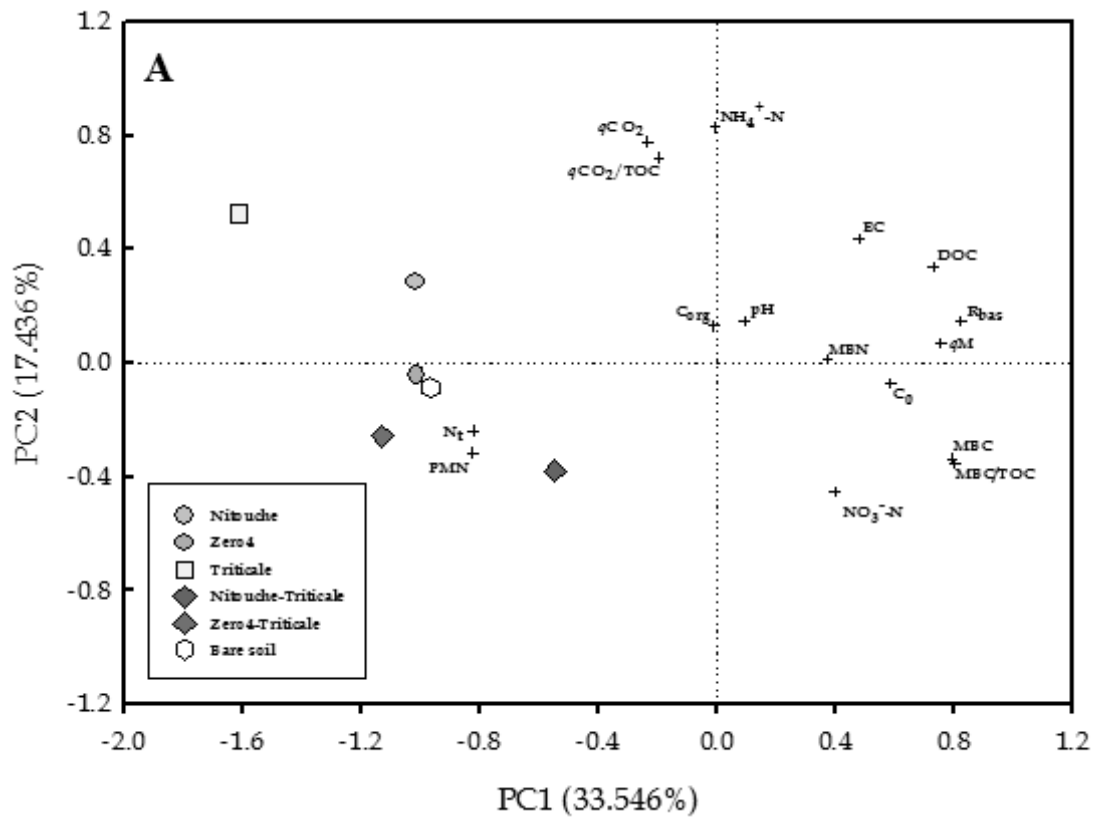


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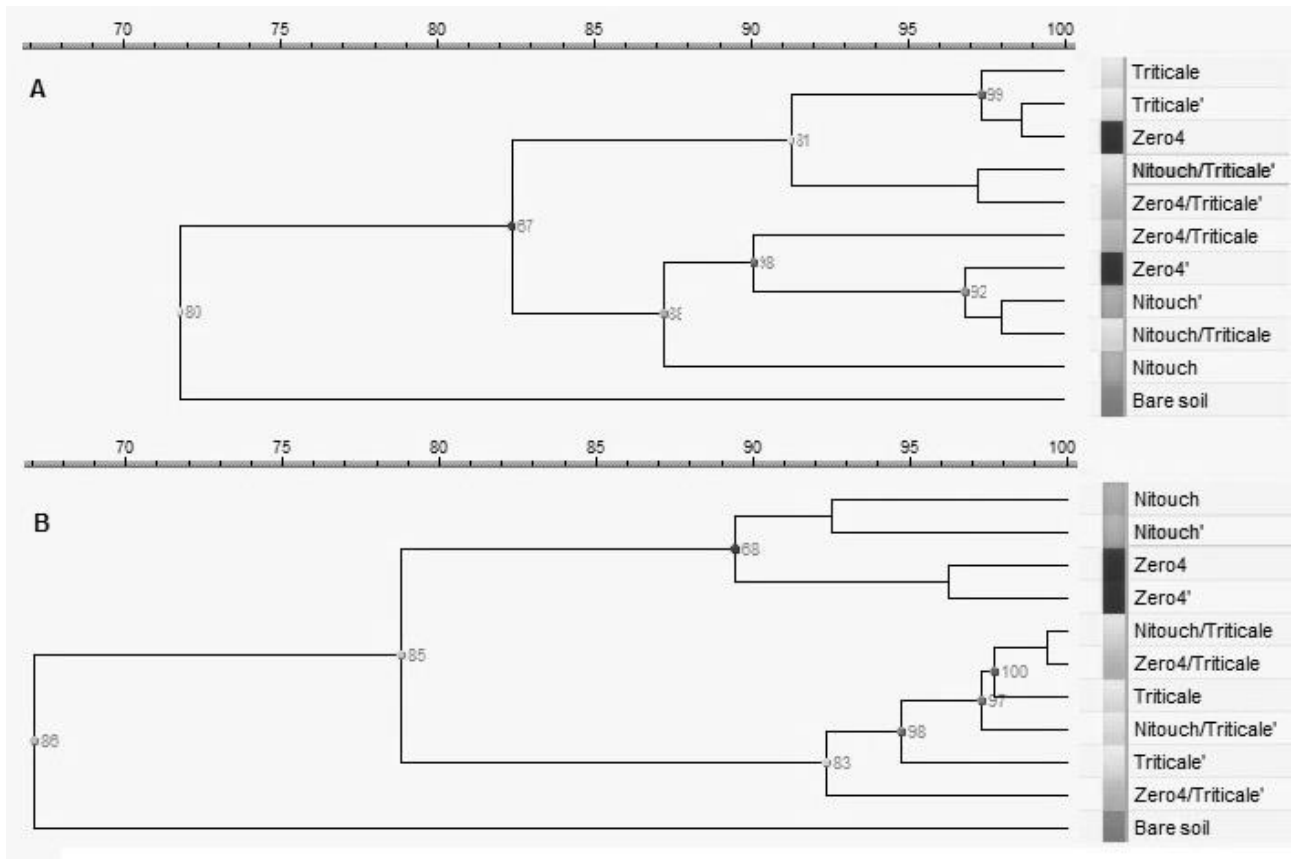


1606 **Figure 4 (PCA analysis)**



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1608 **Figure 5 (ARISA analysis)**



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